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## PREFACE

This volume contains the text of 18 invited papers presented at the USDA Neem Workshop held at Beltsville, Maryland on April 16-17, 1990. The Workshop was organized to bring together researchers, primarily from North America, who are actively involved in neem research. The purpose of this research is to develop natural plant products to reduce dependency on synthetic pesticides. A total of 40 participants, representing federal and university research, private industry, extension, administration, and grower organizations actively contributed to the program. The Workshop ended with several discussion sessions addressing the current status of research, research priorities, and potential cooperation.

The Florist and Nursery Crops Laboratory, Plant Sciences Institute, hosted the Workshop and many people contributed to its success. The organizing committee: Peggy Hall, Dr. H. T. Hsu, Dr. H. G. Larew, Dr. R. H. Lawson, and Judith Thompson, did an outstanding job on local arrangements and hospitality for all of the participants.

We gratefully acknowledge the support of Paul Ecke Poinsettias, Encinitas, California and Yoder Brothers, Inc., Alva, Florida that helped make this Workshop possible.

James C. Locke, Chairman  
Neem Workshop Organizing Committee



## LIST OF PARTICIPANTS

Dr. K. R. S. Ascher	Department of Toxicology Agricultural Research Organization The Volcani Center Bet Dagan, Israel
Dr. Joseph W. Begley	Yoder Brothers, Inc. P. O. Box 68 Alva, Florida 33920
Mr. Clifford Bellande	CARE-Haiti BP 773 Port Au Prince, Haiti
Ms. Jo-Ann Bentz	USDA, ARS, Florist & Nursery Crops Lab. Rm. 10, Bldg. 470, BARC-East Beltsville, Maryland 20705
Dr. Deepak Bhatnagar	USDA, ARS, Southern Region Research Center 1100 Robert E. Lee Blvd. P. O. Box 19687 New Orleans, Louisiana 70179
Dr. John D. Briggs	The Ohio State University Department of Entomology 103 Botany & Zoology Building 1735 Neil Avenue Columbus, Ohio 43210-1220
Mr. Raymond Brush	American Assoc. of Nurserymen, Inc. 1250 I Street, N.W., Suite 500 Washington, D. C. 20005
Dr. Robert M. Faust	USDA, ARS, National Program Staff Rm. 236, Bldg. 005, BARC-West Beltsville, Maryland 20705
Dr. R. G. Gilbert	USDA, SES, ECS P. O. Box 2890 Room 4243, South Building Washington, D. C. 20013
Dr. Kim Hoelmer	USDA, ARS, U. S. Horticultural Research Lab. 2120 Camden Road Orlando, Florida 32803
Dr. Murray B. Isman	Department of Plant Science Suite 248-2527 Main Hall University of British Columbia Vancouver, B. C. Canada V6T 2A2
Mr. Martin Jacobson	1131 University Blvd., West Silver Spring, Maryland 20902
Dr. Andrew P. Jovanovich	Technology Services Group, Inc. 1625 K Street, N.W., Suite 375 Washington, D. C. 20006
Dr. James F. Knauss	Grace/Sierra Horticultural Products 570 Grant Way Fogelsville, Pennsylvania 18051

Dr. Hiram G. Larew	Office of the Science Advisor Rm. 320, SA-18 U. S. Agency for Inter. Development Washington, D. C. 20523
Mr. Robert O. Larson	Vikwood Botanicals, Inc. P. O. Box 4114 Sheboygan, Wisconsin 53082
Dr. Roger H. Lawson	USDA, ARS, Florist & Nursery Crops Lab. Rm. 208, Bldg. 004, BARC-West Beltsville, Maryland 20705
Dr. Zev Lidert	Rohm and Haas Company Spring House Research Center Spring House, Pennsylvania 19477
Dr. Richard K. Lindquist	Department of Entomology Ohio Agricultural R & D Center Wooster, Ohio 44691
Dr. James C. Locke	USDA, ARS, Florist & Nursery Crops Lab. Rm. 208, Bldg. 004, BARC-West Beltsville, Maryland 20705
Dr. Julius J. Menn	USDA, ARS, Plant Sciences Institute Rm. 425, NAL Beltsville, Maryland 20705
Dr. Brian Moss	NPI 417 Wakara Way Salt Lake City, Utah 84108
Dr. John W. Neal	USDA, ARS, Florist & Nursery Crops Lab. Rm. 8, Bldg. 470, BARC-East Beltsville, Maryland 20705
Dr. Ronald D. Oetting	Department of Entomology Georgia Experiment Station Experiment, Georgia 30212
Dr. James F. Price	University of Florida Agricultural Res. and Develop. Center 5007 - 60th Street East Bradenton, Florida 34203
Dr. Edward B. Radcliffe	Department of Entomology University of Minnesota St. Paul, Minnesota 55108
Dr. Roland Seymour	Dept. of Plant Biology The Ohio State University 1735 Neil Avenue Columbus, Ohio 43210-1220
Prof. Heinrich Schmutterer	Institute of Phytopathology and Applied Zoology Justus-Liebig-University D-6300 Giessen, West Germany
Dr. Eugene B. Schultz, Jr.	Prof. of Engineering & Applied Science Washington University St. Louis, Missouri 63130



Dr. Shobha Sriharan	Selma University Division of Natural Sciences Selma, Alabama 36201
Dr. John Stark	USDA, ARS, Kauai Br. Station P. O. Box 1330 Kapaa, Hawaii 96746
Dr. Iroka Udeinya	Department of Immunology Walter Reed Army Institute of Research Washington, D. C. 20307
Dr. Diane E. Ullman	University of Hawaii Department of Entomology 3050 Maile Way Honolulu, Hawaii 96822
Dr. Noel D. Vietmeyer	National Academy of Sciences 2101 Constitution Ave., N.W. Washington, D. C. 20418
Dr. James F. Walter	W. R. Grace & Co.-Conn 7379 Route 32 Columbia, Maryland 21044
Dr. J. David Warthen	USDA, ARS, Insect Chem. Ecol. Lab Rm. 120, Bldg. 001, BARC-West Beltsville, Maryland 20705
Mr. Peter Welle	CARE-Haiti BP 773 Port Au Prince, Haiti
Dr. Tim Wood	NPI 417 Wakara Way Salt Lake City, Utah 84108
Dr. Geoffrey W. Zehnder	Virginia truck & Ornamental Research Station Rt. 1, Box 133 Painter, Virginia 23420
Dr. Eldon I. Zehr	Department of Plant Pathology and Physiology Clemson University Clemson, South Carolina 29634-0377

## SUMMARY

Pest management in the U.S. has taken a dramatic turn during the 1980's. As synthetic pesticide registrations are being withdrawn, and pests are becoming resistant to the remaining products, interest has been renewed in botanical pesticides. The USDA, ARS has taken an active role for the past 15 years in pioneering research on compounds from the neem tree (*Azadirachta indica* A. Juss). Interest in this source of botanical pesticides has resulted in three international conferences. This Workshop was organized to provide an informal setting for North American researchers to exchange their recent findings and to discuss research priorities, registration possibilities, and the potential for cooperation. This Proceedings is designed to provide a current update on the status of neem research in North America.

In the opening session, Dr. J. Menn described the USDA interest in natural products. He emphasized that research on potentially promising botanicals such as neem is important in ARS pest control research. Dr. M. Jacobson reviewed the history of neem research in the U.S. including: cultivation, chemistry, biological activity, and pharmacology. Dr. H. Schmutterer concluded this session with his perspective on worldwide agricultural production needs and the role that neem research can play in addressing problems of pest resistance, environmental pollution, and the negative impact of synthetic pesticides on natural enemies of pests.

The session on commercialization of neem-based products included three presentations which addressed the efficacy of neem, patenting and securing the initial EPA registration for Margosan-O. Robert Larson related his personal experiences of dealing with seed supply, formulation, toxicology, and ultimately the process of registration for a product of "unique formulation and chemical type". Drs. Walter and Knauss discussed the key steps that W. R. Grace & Co. utilized in bringing Margosan-O to the market place. These steps included market analysis, formulation development, cooperative network evaluation, commercial field testing, and marketing. They stressed that botanical extract products present unique problems as well as opportunities in development. Dr. M. Isman covered the development process for a neem-based product in Canada. This involved extensive laboratory and field testing, as well as chemical evaluation of neem oil sources and mode of action studies. He concluded that neem-based products will have the greatest impact in the domestic use (home and garden) and "organic" agriculture markets. Additional improvements in neem product performance will be required before synthetic insecticides are displaced. However, increased public demand for more environmentally-sound pest control materials will result in an increased market for these materials in the 1990's Isman predicted.

Field evaluations of neem were reported by Drs. Ascher, Radcliffe, and Zehnder in a session that focused on applied aspects including comparative studies with pyrethroid insecticides. Dr. Ascher's presentation (kindly delivered by Dr. Schmutterer in the author's absence) described the activity of various solvent extracts of neem seed kernels on insects and mites harmful to field crops. Generally, extracts made with more polar solvents had more activity against insects but the reverse was true with spider mites. He concluded that the excitement generated in the past few decades about the pest control possibilities of neem is a classical example of so-called "recent technological innovations" that have in fact been well established in some cultures for centuries. Dr. Radcliffe reported on studies involving pyrethroid-resistant Colorado potato beetles on potato in Minnesota. He found that when the larvae

hatched on neem-treated foliage there was essentially no survival but larvae established prior to treatment were not adequately controlled. Thus, timing of application is critical and tank mixing neem with a conventional insecticide (for the first application) or with a synergist (piperonyl butoxide) may improve efficacy. Dr. Zehnder concurred that the efficacy of neem depends on the life stage of Colorado potato beetle, with the greatest effect against young larvae. He suggests that limited use of neem products, targeted at early season small larvae, and rotation with other effective insecticides will prolong the effective life of these new botanicals.

The session focusing on laboratory and greenhouse evaluations of neem included reports on: mode of action, life stage susceptibility, application method, impact on beneficial insects, and efficacy against pyrethroid-resistant insects. Dr. T. Wood discussed the antifeedant and growth regulator activities of several formulations of neem extracts, indicating that from his studies the growth regulator activity appears to be of greater commercial significance. Dr. R. Lindquist reported that effectiveness of the neem extract, Margosan-O, in control of greenhouse whitefly depended on spray application to the abaxial leaf surfaces. He also noted that both bifenthrin-resistant and susceptible whiteflies were controlled and that there were sublethal effects on larvae resulting in delayed adult emergence. Results of neem extract evaluations with predators and parasitoids of the cotton aphid were presented by Dr. K. Hoelmer. He found that neem extracts were relatively non-toxic to the beneficial insects and did not greatly reduce predation and parasitism, suggesting that these extracts are compatible with the use of natural enemies in the greenhouse. Dr. J. Stark concluded this session with a discussion of the effects of neem seed extract on the life cycles of three fruit flies, development of fruit fly parasitoids, and neem's potential for use in integrated control programs. His results indicate that soil applied treatments of neem extract can be effective in suppressing tephritid fly populations and that fruit fly parasitoids can develop in neem treated hosts giving promise for its use in IPM programs.

The final session included four presentations describing novel uses of neem extracts. Dr. R. Oetting described the use of neem seed extract as a chrysanthemum cutting treatment; before, during, and after shipment, to reduce leafminer populations. This was accomplished by either basal stem soaks or vacuum infusion. Both methods were effective in interrupting the development of leafminers. Dr. D. Bhatnagar evaluated neem leaf extracts against fungi that produce toxic secondary metabolites (aflatoxins). Although the extracts did not inhibit vegetative fungal growth either *in vitro* or *in vivo*, the production of aflatoxins was blocked in the early steps of biosynthesis. This research may lead to preharvest procedures to reduce aflatoxin contamination of food and feed commodities. Dr. H. Larew introduced the potential use of another neem seed extraction product, neem oil. He presented evidence that neem oil is both toxic and repellent to several common greenhouse insects, including ovicidal and nymphicidal activity. Finally, Dr. J. Locke reported the activity of extracted neem oil against several foliar fungal pathogens. Neem oil formulated with an emulsifying agent provided excellent protection of plant foliage from rust and powdery mildew infection. Application of aqueous sprays of as little as 0.5% oil consistently gave excellent protection of bean foliage from rust. These results may lead to development of botanical fungicides as an important component in disease management and reduce dependence on synthetic fungicides.



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## Overview of Neem Research

### USDA INTEREST IN NEEM RESEARCH: PAST AND PRESENT //

Julius J. Menn

Insect control through the use of natural products is almost as old as the recorded history of mankind. The prophet Joel graphically described the ravages brought on by the desert locust: "...their vanguard a devouring fire, their rearguard a leaping flame, before them the land is a garden of Eden, behind them a wasted wilderness...."

References to locust invasions and use of burning fields as a control measure were also recorded in China during the Shang Dynasty (1520 to 1030 B.C.).

In recent times, Gill and Lewis (1971) reported that azadirachtin isolated from the neem tree Indian lilac, was apoplastically transported in plants, providing systemic phagorepellency against the desert locust, *Schistocerca gregaria*. Most likely this is the same locust referred to in the Bible and Chinese chronicles. The structure of azadirachtin was first reported by Zanno et al. (1975) and corrected in the publication by Bilton, et al. (1987).

This complex triterpenoid is probably the most potent insect antifeedant reported to date. Natural products research targeted to discovery and development of safe, selective and environmentally compatible insect control agents already started in the USDA in the late 1920's. C. R. Smith embarked on a synthesis program for nicotine analogs that resulted in the synthesis of anabasine, at that time proving to be a potent aphicide.

USDA scientists, F. B. LaForge, W. F. Barthel, and M. S. Schechter were pioneers in the synthesis of synthetic pyrethroids such as allethrin and barthrin in the 1940's that laid the chemical foundation for Elliott and Janes at the Rothamsted Experimental Station in England (Elliott et al., 1973) to synthesize the first more photostable pyrethroids. Unfortunately, the chemical research to optimize synthetic pyrethroids in ARS terminated due to a policy decision in 1954 after the successful commercial introduction of allethrin (Klassen and Schwartz, 1985). Nevertheless, ARS research on natural product optimization left its mark, as acknowledged by the British group headed by Michael Elliott and others, that it was allethrin that paved the way for syntheses of photostable pyrethroids.

In subsequent years, ARS emphasized again in its mission the discovery and development of natural control agents useful in biocontrol and integrated pest management (IPM) programs in crop protection and in protecting man and animals from disease-carrying and nuisance insects, ticks and mites.

Most notable successes were recorded in the discovery and introduction into use of numerous semiochemicals including pheromones and attractants useful in monitoring and control programs.

Research on neem extracts started in ARS in 1975 in the Biologically Active Natural Products Laboratory (presently the Insect Chemical Ecology Laboratory), Beltsville Agricultural Research Center under the direction of Martin Jacobson (Jacobson, 1981). This laboratory served as the chemical focus for neem research in cooperation with 15 ARS laboratories primarily engaged in bioevaluation of a variety of insectostatic and insecticidal properties of neem extracts.

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Associate Deputy Area Director, {USDA, ARS, Plant Sciences Institute,  
Beltsville, Maryland} 20705

Research in these laboratories established the exquisite activity of azadirachtin as a phagorepellent or toxicant against a variety of insects in the 0.1 to 1000 ppm range when incorporated into diets. No other feeding repellent has every equaled this activity.

Warthen and co-workers (1984) developed a very useful HPLC method for estimating the azadirachtin content of neem extracts and formulations.

In another effort, ARS also embarked on experimental cultivation of neem trees in Florida, Puerto Rico, and the Virgin Islands, and on research to determine optimal physiological and environmental parameters affecting cultivation of neem (Jacobson, 1987).

Recently, Larew (1987) demonstrated the efficacy of neem seed kernel extract (NSKE) applied to the soil, to control *Liriomyza* leafminer larvae in ornamental croppings. ARS supported these studies with pilot test funds under field conditions confirming the utility of this treatment in a practical setting.

Currently, a concerted collaborative research effort is underway between scientists in the Florist and Nursery Crops Laboratory and scientists in several other laboratories in the Plant Sciences Institute, Beltsville Agricultural Research Center and industry to evaluate the pesticidal potential of a comprehensive group of neem fractions not only for their insecticidal properties but also for potential application to control plant pathogens and phytoparasitic nematodes.

Research on neem extracts and other potentially promising natural products is very much in the mainstream of the ARS mission to develop pest control agents based on natural products that harmonize well with the principles of integrated pest management.

## REFERENCES

- Bilton, J. N., Broughton, HJ. B., Jones, P. S., Ley, S. V., Lidert, Z., Morgan, E. D., Rzepa, H. S., Sheppard, R. N., Slawin, A. M. Z. and Williams, D. J. 1987. An X-ray crystallographic, mass spectroscopic and NMR study of the limonoid insect antifeedant azadirachtin and related derivatives, *Tetrahedron* 43:2805.
- Elliot, M., Farnham, A. W., Janes, N. F., Needham, P. H., Pullam, D. A., and Stevenson, J. H. 1987. A photostable pyrethroid. *Nature, Lond.* 246:169-170.
- Gill, J. S., and Lewis, C. T. 1971. Systemic action of an insect feeding deterrent. *Nature, Lond.* 232:402-403.
- Jacobson, M. 1981. Neem research in the U. S. Department of Agriculture: Chemical, biological and cultural aspects. In: *Proc. First International Neem Conf.*, Rottach-Egern, FRG, Schmutterer, H., Ascher, K. R. S., and Rembold, H. Eds., GTZ Press, 1981, pp. 33-42.
- Jacobson, M. 1987. Neem research and cultivation in the Western Hemisphere. In: *Proc. Third International Neem Conf.*, Nairobi, 1987, pp. 33-44.
- Klassen, W., and Schwartz, P. H. 1985. ARS research program in chemical insect control. pp. 267-291, In: J. E. Hilton (Editor) *Agricultural Chemicals of the Future*, Rowman and Allanheld, Totowa, Iowa.



Larew, H. G. 1987. Use of neem seed kernel extract in a developed country: Liriomyza leafminer as a model case. In: Proc. Third International Neem Conf., Nairobi, Schmutterer, H., and Ascher, K. R. S., Ed., 1986, pp. 375-385.

Warthen, J. D., Jr., Stokes, J. B., Jacobson, M., and Kozempel, M. F. 1984. Estimation of azadirachtin content in neem extracts and formulations. J. Liquid Chromatog. 7:591-598.

Zanno, P. R., Miura, L., Nakanishi, K. and Elder, D. L. (1975). Structure of the insect phagorepellent azadirachtin. Application of PRFT/CWD carbon-13 nuclear magnetic resonance. J. Amer. Chemical Soc. 97:1975-1977.

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REVIEW OF NEEM RESEARCH IN THE UNITED STATES //

M. Jacobson

ABSTRACT

In 1974-75, a group at Columbia University in New York reported the chemical identification of portions of the azadirachtin and salannin molecules responsible for the major pesticidal properties of neem seeds. Also in 1975, the U.S. Department of Agriculture (USDA) station at Beltsville, MD and 10 USDA stations in this country embarked on a comprehensive research program on silviculture, chemistry, and pest control properties of neem. Several American universities collaborated or worked independently on this program. The research, which still continues at several locations, has demonstrated or verified the outstanding effects of neem extracts against numerous species of destructive insect and fungal pests. The tree is being successfully grown at USDA Experimental Stations in Miami, FL, Mayaguez, Puerto Rico, and St. Croix, U.S. Virgin Islands. The commercialization of neem formulations in the U.S. is a reality.

INTRODUCTION

Although a considerable amount of research (mainly biological) has been done with neem in India during the first half of the twentieth century, it was not until 1974-75 that Nakanishi and his group at Columbia University in New York reported on their chemical research with azadirachtin (Zanno, 1974; Nakanishi, 1975), the major insecticidal component of neem seeds and fruits, resulting in the chemical identification of major portions of this compound and of the closely related salannin. In 1975, the Biologically Active Natural Products Laboratory of USDA at Beltsville, embarked on a comprehensive neem program encompassing the chemical and biological research that involved scientists in numerous USDA laboratories on the mainland, Puerto Rico, and the Virgin Islands (Jacobson, 1987). A number of universities as well as commercial and Government organizations worked independently or collaborated on the neem program, reporting new and improved methods for the isolation and identification of numerous components present in various parts of this wonder tree and their pesticidal properties against many species of insect pests of garden and field crops, man and animals, as well as diseases caused by microorganisms and fungi. This research has demonstrated or verified the outstanding effects of neem extracts against various species of destructive pests.

CULTIVATION

In 1979 the Biologically Active Natural Products Laboratory of USDA-ARS at Beltsville enlisted the aid of USDA Agricultural Experiment Stations at the Fairchild Tropical Garden in Miami, FL, the Horticultural Research Station at Mayaguez, Puerto Rico, and the Agricultural Experiment Station at St. Croix, U.S. Virgin Islands. One neem tree brought from Africa was replanted in Miami. Two trees brought from Malaysia were replanted in Mayaguez. Neem trees were also grown from seed at all three stations (Jacobson, 1981). Since dry or unripe seeds tended to rot if planted in soil, they were planted in sand over a layer of pebbles and irrigated with water dripped into the trays. Complete germination was obtained using this method in Puerto Rico. When the seedlings were one month old they were transplanted to clay pots, and when the seed had achieved a height of 30-45 cm they were planted in the field (Jacobson et al., 1984). This method was later used in Florida and the Virgin Islands. All neem trees at the three locations are doing very well at the present time. According to Pliske (1984), 30 neem seedlings from Indonesia were planted at the Tamiami Campus of Florida International University.

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Jacobson is a research chemist collaborator with the Insect Chemical Ecology Laboratory, Plant Sciences Institute, USDA-ARS, Beltsville, MD 210705

Neem trees are currently under cultivation in this country in Arizona, Florida, California, and Oklahoma, in Puerto Rico, and in Kingshill, U.S. Virgin Islands (Jacobson, 1987). The trees at all locations continue to develop well. Those trees in Arizona, California, and Oklahoma were all begun by private organizations or local farmers. Approximately 50 trees are growing in the field in southern California after a triple transplant in 1984. In Arizona, the acreage devoted to neem was established with the goal of developing a neem tree that will be frost-tolerant to  $-18^{\circ}\text{C}$ , using seed from northern India where the climate is cooler than in most areas where neem grows.

## CHEMISTRY

Although a large number of terpenoidal compounds have been isolated from various parts of the neem tree, only three (azadirachtin, salannin, and meliantriol) have been shown to be highly active as pesticides (Jones et al., 1989). The most active and versatile of these compounds is azadirachtin; for this reason its isolation and structural determination have received much attention. However, all attempts to obtain this compound in other than amorphous form failed until 1987, when a group of scientists at Columbia University isolated the pure compound as white microcrystals, m.p.  $149^{\circ}$  (Schroeder and Nakanishi, 1987). The yield of pure azadirachtin was slightly more than 5 g from 2 kg of seeds. The isolation procedure involved extraction of the seeds with 95% ethanol, partition of the extract between 95% methanol and petroleum ether, partition of the methanolic extract between ethyl acetate and water, silica gel filtration of the organic layer with ethyl acetate, liquid chromatography using ethyl acetate-hexane (3:1), crystallization of the azadirachtin fraction from chloroform, and flash chromatography using chloroform-acetonitrile (3:1). The prior isolation method used by the Columbia University group had been described by Zanno (1974) in his Ph. D. dissertation and by Nakanishi (1975).

Several modifications of Zanno's procedure were used by the USDA Biologically Active Natural Products Laboratory to isolate amorphous azadirachtin of greater than 90% purity (Warthen et al., 1978; Jacobson et al., 1978; Uebel et al., 1979; Kubo and Nakanishi, 1979), and by Lee et al. (1986) at Native Plants Incorporated (NPI) in Salt Lake City, Utah.

A structural reappraisal of azadirachtin was offered in 1985 by two British groups with the assistance of Dr. Zev Lidert of the Rohm & Haas Co., Spring House, PA (Bilton et al., 1985) and more recently by investigators at NPI (Yamasaki and Klocke, 1987). A review of terpenoid insect antifeedants has been published (Kubo and Nakanishi, 1979).

Since all indications pointed to the extreme difficulty (and possible inability) of synthesizing the complicated azadirachtin structure, the synthesis of a number of compounds with modifications of the azadirachtin molecule was carried out by several groups. The importance of the tigloyl group in azadirachtin to high pesticidal activity and the close relationship of the molecule to steroids prompted the preparation of the tiglic esters of cholesterol and sitosterol at Beltsville, when tested for antifeedant activity on larvae of the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) and the southern corn rootworm, *Diabrotica undecimpunctata* Howard (Barber) (Jacobson, 1987). A compound with structure related to that of azadirachtin was isolated by a group at the University of California at Berkeley in collaboration with NPI; it proved to be deacetylazadirachtinol (Kubo et al., 1984) and was deterrent to feeding by larvae of the tobacco budworm, *Heliothis virescens* (Fabricius) (Kubo et al., 1986). The compound 7-deacetyl-17-hydroxyazadiradione was isolated from neem by a group at NPI and the University of Utah (Lee et al., 1986). Other compounds isolated were 22,23-dihydroazadirachtin, 2<sup>1</sup>, 3<sup>1</sup>, 22, 23-tetrahydroazadirachtin, and 3-deacetylazadirachtin (Barnby and Klocke, 1987; Barnby et al., 1989). Nimbolide and 28-deoxonimbolide were isolated by investigators at the

University of Chicago, IL (Kigodi et al., 1989). Salannin was isolated by scientists at the University of Maine, Orono (Rajab et al., 1988) and at NPI (Yamasaki and Klocke, 1989). Salannin proved to be a feeding deterrent for the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) Yamasaki and Klocke, 1989). It was converted synthetically to several derivatives that were even more effective against this insect than salannin itself. USDA scientists at Beltsville devised a method for estimating the azadirachtin content of neem extracts and formulations (Warthen et al., 1984).

## BIOLOGICAL EFFECTS

### Field Crop Insects

Incorporation of an ethanol extract of neem seeds into the diet at 0.116  $\mu\text{l/ml}$  was highly toxic to neonate larvae of the beet armyworm (*Spodoptera exigua* Hübner). These tests were conducted at the University of California, Riverside (Moab and Trumble, 1987). In tests conducted at the USDA Fruit and Vegetable Insects Laboratory, incorporation of the extract at 0.02-2.0% prolonged the development of and induced mortality in all larval stages; none of the larvae pupated (Prabhaker et al., 1986).

Tests conducted in a greenhouse at USDA Beltsville (Redfern et al., 1984) using an ethanol extract of the seeds as an aqueous suspension applied to sweet corn exposed to 1st stage larvae for 23 days failed to elicit feeding by fall armyworm (*Spodoptera frugiperda* [J. E. Smith]) larvae. Application as a dust was somewhat less effective. An artificial diet containing a hexane or ethanol extract of the seeds was fed upon by larvae that were either rapidly killed or showed reduced growth, in tests conducted at the USDA Northern Regional Research Laboratory, Peoria, IL (Mikolajczak et al., 1989). Incorporation of azadirachtin (at least 90% pure) into the diet at 0.35 ppm was highly deterrent to feeding by 1st instar larvae and caused severe weight loss in tests conducted at USDA Beltsville laboratories (Warthen et al., 1978); incorporation into the diet at 0.2 ppm was also satisfactory. Larvae treated topically with 10 g quickly perished; this was also true of the large milkweed bug (*Oncopeltus fasciatus* (Dallas)) (Redfern et al., 1981). Azadirachtin and salannin, supplied to Israeli scientists by USDA and applied to cotton leaves at 0.001% and 0.005%, respectively, repelled larvae of *S. littoralis* and *Earias insulana* (Boisd.) (Meisner et al., 1981).

Tests conducted at the University of California at Berkeley showed that nomilin (a neem limonoid) applied to cotton leaf disks was 10-fold more active as a feeding deterrent for newly molted 3rd instar larvae of *S. frugiperda* than for larvae of the corn earworm (*Heliothis zea* [Boddie]). Azadirachtin in a choice test was highly active as an antifeedant and was a potent toxicant at concentrations below 10 ppm (ecdysis inhibition) (Klocke and Kubo, 1982). In a no-choice situation, these species as well as larvae of the pink bollworm (*Pectinophora gossypiella* [Saunders]) and of the tobacco budworm (*Heliothis virescens* [Fabricius]) refused to feed (Kubo and Klocke, 1982).

In tests conducted by scientists at NPI azadirachtin incorporated into the diet at 0.03125 ppm deterred feeding and reduced weight gain by 5th instar *H. virescens* larvae. Oral injection at 0.25 or 0.5  $\mu\text{g}$  delayed molting to the pupal stage, produced defective pupae or adults, and inhibited development to the adult stage (Barnby and Klocke, 1987). Larvae injected on the first day of the 5th instar failed to pupate. Azadirachtin, 22,23-dihydroazadirachtin, 21, 31, 22,23-tetrahydroazadirachtin, and 3-deacetylazadirachtin injected at 0.1  $\mu\text{g}$  prevented pupation. The 21,31,22,23-derivative was highly stable to ultraviolet light after 200 hr of exposure, whereas the other derivatives were unstable (Barnby et al., 1989). Deacetylazadirachtinol obtained from neem oil was as potent as azadirachtin in inhibiting ecdysis when fed to *H. virescens* larvae. Salannin, 3-desacetylsalannin, and 6-O-acetylnimbendiol, which was also isolated from the oil, was much less active (Kubo and Nakanishi, 1979).



Azadirachtin applied to bean leaves at 19.8 ug/sq cm strongly deterred feeding by larvae of *H. virescens* and of the southern armyworm (*Spodoptera eridanea* [Cramer]) and Mexican bean beetle (*Epilachna varivestis* Mulsant); the effect was much weaker at 1.5 ug and 6 ug. These tests were conducted by scientists at Rohm and Haas (Lidert et al., 1985).

Pener et al., (1988), working in Israel with azadirachtin supplied by Beltsville, injected various doses of this compound dissolved in 90% ethanol into larvae of the tobacco hornworm (*Manduca sexta* L.). The nearly normal pupae obtained were then injected with the solution, which stimulated in vitro ecdysone production by the prothoracic glands.

Laboratory and greenhouse tests were conducted with aqueous solutions of neem seed extracts at the USDA Riverside station in an attempt to control the sweetpotato whitefly, *Bemisia tabaci* (Gennadius). Cotton foliage treated with 0.2% and 0.3% solutions and exposed to the insects caused a reduction in egg viability and oviposition, prolonged larval periods, and killed the larvae. Larvae treated topically with the solution failed to complete ecdysis (Coudriet et al., 1985).

In both laboratory and field tests conducted over a three-year period at USDA stations at Beltsville and in Wooster, Ohio, beginning in 1975, scientists found that aqueous formulations prepared from ethyl ether or methanol extracts of neem seeds deterred Japanese beetles (*Popillia japonica* [Newman]) from feeding on sassafras and soybean leaves. Untreated foliage was completely destroyed (Ladd et al., 1978, 1979; Ladd, 1981). Topical application of azadirachtin (0.16 g in acetone) to the larvae completely disrupted normal development to the adult stage and increased the duration of the immature stages (Ladd, 1981; Ladd et al., 1984). Seventeen Kwansan cherry trees growing around the Tidal Basin in Washington, D.C. were sprayed by the U. S. Department of the Interior with a 1% aqueous emulsion of a crude ethanol extract of neem kernels. Although the trees had previously been attacked each year by Japanese beetles, the damage following treatment was held to only 2.4% leaf loss on side branches, compared with 35.5% loss on nearby unsprayed trees (Jacobson, et al., 1984).

An ethanol extract of neem seeds provided by USDA scientists at Beltsville was sprayed on potato leaves at 0.8%, 1%, and 10.4% by scientists at Virginia Polytechnic Institute (Zehnder and Warthen, 1988). Feeding by adults and larvae of the Colorado potato beetle (*Leptinotarsa decemlineata* [Say]) was inhibited. The effects were enhanced by adding piperonyl butoxide at the concentration of 10:1. Spray applications at 1.2% were effective in the field. Fourteen derivatives of salannin were prepared by NPI and tested against 3rd instar beetle larvae; several of the derivatives were at least 40-fold more active than salannin (Yamasaki and Klocke, 1989).

Greenhouse and seedbed tests conducted at the Vincennes, IN station of USDA showed that ethanol extracts of neem seeds prepared at Beltsville strongly prevented feeding by adult striped cucumber beetles (*Acalymma vittatum* [Fabricius]) and spotted cucumber beetles (*Diabrotica undecimpunctata howardi* [Barber]). Greenhouse and seedbed tests had previously proved to be highly repellent to the adults of both species when adsorbed on cantaloupe leaves (Reed and Reed, 1981; Reed et al., 1982). Ethanol extracts of neem seeds strongly prevented feeding by *A. vittatum* and fall armyworms (*Spodoptera frugiperda* [Mikolajczak and Reed, 1987]).

High concentrations (5% and 10%) of Margosan-0 applied in the laboratory to cotton leaf squares were necessary to significantly reduce feeding by mixed sexes of adult Carolina grasshoppers (*Dissosteira carolina* [L.]), walkingsticks (*Diaperomera femorata* [Say]), and field crickets (*Gryllus pennsylvanicus* [Burmeister] [Adler and Uebel, 1984]). Newly hatched nymphs of house crickets (*Acheta domesticus* (L.)) fed a diet containing 1, 10, or 25 ppm azadirachtin gained little weight and showed little development. Topical application of 50 ug azadirachtin to 7th instar nymphs killed a large number of the insects prior to molting; 75 ug applied to 8th instars prevented the crickets from shedding their cuticles, causing death (Warthen and Uebel, 1981).

In Israel, Pener and Shalom (1987) injected azadirachtin obtained from Beltsville into 5th instar hoppers of the migratory locust (*Locusta migratoria migratorioides* [R. & F.]) which failed to molt to adults and the treated males showed subnormal sexual behavior.

A cooperative endeavor between the Livestock Insects Laboratory and the Insect Reproduction laboratory at USDA Beltsville found that ecdysteroid production was delayed and erratic in last-stage nymphs of the large milkweed bug (*Oncopeltus fasciatus* [Dallas]) treated topically on the last abdominal segment with an acetone solution of azadirachtin at 10 ug/nymph (Redfern et al., 1982).

In 1983 the Department of Entomology at Ohio State University (Wooster) conducted a collaborative project with the USDA Florist and Nursery Crops Laboratory (Beltsville) to control leafminers. Chrysanthemum plants in the greenhouse were sprayed with a 1% solution of an ethanol extract of neem seed. Adult *Liriomyza trifolii* (Burgess) were repelled for 7 days, the larvae that did develop died quickly, and fewer eggs were laid (Lindquist et al., 1983). Stein (1984) showed conclusively that the leafminers were repelled by the formulation. Application of the seed extract at 0.1% to the soil of potted plants gave good control of *L. trifolii* and of the vegetable leafminer (*L. sativae* [Blanchard]) for at least 3 weeks; the extract, as well as azadirachtin applied to lima bean plants at 0.2% were highly effective for controlling the larvae. Extract applied to the soil was translocated to the foliage of the plants (Larew et al., 1984; Webb et al., 1983).

When applied as a soil drench to bed-grown chrysanthemum plants, 0.4% neem seed extract and 0.33% Margosan-0 killed *L. trifolii* larvae and pupae in an infested commercial greenhouse by disrupting the insect's life cycle (Larew et al., 1985; Larew, 1987). Margosan-0 applied at 0.17% or 0.33% to soil in pots of chrysanthemums or marigolds (but not zinnias) sharply reduced the number of adult leafhoppers (Knodel-Montz et al., 1985); as a 0.33% soil drench applied to chrysanthemum plants, the number of *L. trifolii* pupae and adults was reduced for at least 21 days. As a foliar spray, concentrations of 0.41%, 0.84%, and 1.25% of Margosan-0 reduced the number of adult leafminers without inhibiting plant growth (Knodel et al., 1988). A 1% solution of neem seed extract applied against *L. trifolii* failed to increase the toxicity of untreated chrysanthemum foliage positioned above or below sprayed foliage. Untreated bean leaves that were opposite leaves painted with 0.4% extract were not as effective as painted leaves (Larew, 1988).

A 1% neem seed extract applied as a foliar spray against the birch leafminer (*Fenusa pusilla* (Lepetier)) was compared with sprays of water and Metasystox insecticide (Larew et al., 1987). The extract was as effective as the insecticide for causing mortality but it required a longer period of time.

### Pests of Man and Animal

Warthen et al. (1978) isolated salannin from an ethanol extract of neem seeds and found that sucrose treated with the compound at 0.1% deterred feeding by adult house flies (Musca domestica [L.]). Highly dilute solutions of azadirachtin in aqueous acetone exposed to 3rd instar face flies (Musca autumnalis [De Geer]) killed many of the larvae and those that survived to adulthood showed inhibited development, reduced reproductive potential, and reduced egg production. Some of the adults were deformed (Gaaboub and Hayes, 1984).

Azadirachtin evaluated at USDA Gainesville, FL for repellency to mosquitoes (Aedes aegypti [L.]) on cloth caused little or no repellent effect at 1 ug/sq cm (Weidhaas, 1978). Tests conducted at Cornell University (Ithaca, NY) showed that azadirachtin contained in blood fed to adult female mosquitoes over a wide dose range (0-200 mg/female) did not inhibit feeding. High doses failed to inhibit or delay oviposition, but retardation of oocyte growth was observed for up to 72 hr after feeding (Ludlum and Sieber, 1988).

Margosan-0 was tested at USDA Beltsville for its effects as a toxicant, growth inhibitor, or repellent against the following species of cockroaches: oriental cockroach (Blatta orientalis [L.]), German cockroach (Blattella germanica [L.]), American cockroach (Periplaneta americana [L.]), Byrsotria fumigata (Guerin-Meneville), Gromphadorina portentosa (Schau), and the brownbanded cockroach (Supella longipalpa [Fabr.]) (Adler and Uebel, 1985). Last instar nymphs of these roach species fed Lab-Chow pellets impregnated with 0.5 ml of Margosan-0 showed a higher rate of mortality than those fed untreated pellets; survivors showed retarded development. Treated pellets fed to the nymphs were completely lethal. Topical applications of 2 ul to the abdomen of last instar B. orientalis nymphs, as well as injection of 0.5 ul, caused retarded growth and increased mortality (Adler and Uebel, 1985).

### PHARMACOLOGY

A frozen lyophilized aqueous extract of neem leaves was studied at the University of Illinois, Urbana, IL for its effects on the cardiovascular system of anesthetized guinea pigs and rabbits (Thompson and Anderson, 1978). Injection of 5-200 mg/kg into the blood stream caused profound hypotension and a minimum negative chronotropic effect.

In vitro evaluation of the neem limonoids, 7-acetylneotrichilenone and T,2-diepoxyazadiradione against the murine P366 lymphocytic leukemia cell line showed that only the former compound was somewhat effective, with an ED<sub>50</sub> of 8.5 ug/ml. The research was conducted by Pettit et al. (1983) at Arizona State University, Tempe, AZ.

### COMMERCIALIZATION

Commercialization of neem in the United States will be discussed by others at this workshop.

## REFERENCES

- Adler, V. E., and E. C. Uebel, 1984. Antifeedant bioassays of neem extract against the Carolina grasshopper, walkingstick, and field cricket. *J. Environ. Sci. Health* A19:393-403.
- Adler, V. E., and E. C. Uebel, 1985. Effects of a formulation of neem extract on six species of cockroaches (Orthoptera:Blaberidae, Blattidae and Blattellidae). *Phytoparasitica* 13:3-8.
- Barnby, M. A., and J. A. Klocke, 1987. Effects of azadirachtin on the nutrition and development of the tobacco budworm, Heliothis virescens (Fabr.)(Noctuidae). *J. Insect Physiol.* 33:69-75.
- Barnby, M. A., R. B. Yamasaki, and J. A. Klocke, 1989. Biological activity of azadirachtin, three derivatives, and their ultraviolet radiation degradation products against tobacco budworm (Lepidoptera:Noctuidae) larvae. *J. Econ. Entomol.* 82:58-63.
- Bilton, J. N. and others (7 coauthors), 1985. Structural reappraisal of the limonoid insect antifeedant azadirachtin. *J. Chem. Soc. Chem. Commun.* 968-971.
- Coudriet, D. L., N. Prabhaker, and D. E. Meyerdirk, 1985. Sweetpotato whitefly (Homoptera: Aleyrodidae):effects of neem-seed extract on oviposition and immature stages. *Environ. Entomol.* 14:776-779.
- Gaaboub, I. A. A., and D. K. Hayes, 1984. Biological activity of azadirachtin component of the neem tree inhibiting molting in the face fly, Musca autumnalis De Geer (Diptera:Muscidae). *Environ. Entomol.* 13:800-812.
- Gaaboub, I. A. A., and D. K. Hayes, 1984. Effect of larval treatment with azadirachtin, a molting inhibitory compound of the neem tree, on reproductive capacity of the face fly, Musca autumnalis De Geer (Diptera:Muscidae). *Environ. Entomol.* 13:1639-1643.
- Jacobson, M., D. K. Reed, M. M. Crystal, and others, 1978. Chemistry and biological activity of insect feeding deterrents from certain weed and crop plants. *Entomol. Exp. Appl.* 24:448-457.
- Jacobson, M., 1981. Neem research in the U.S. Department of Agriculture: chemical, biological and cultural aspects. p. 33-42. In: H. Schmutterer, K. R. S. Ascher, and H. Rembold (eds.), Natural Pesticides From the Neem Tree, GTZ Press, Eschborn, FRG.
- Jacobson, M., J. B. Stokes, J. D. Warthen, Jr., and others, 1984. Neem research in the U.S. Department of Agriculture, An update. p. 31-42. In: H. Schmutterer and K. R. S. Ascher, (eds.), Natural Pesticides From the Neem Tree, GTZ Press, Eschborn, FRG.
- Jacobson, M., 1987. Neem research and cultivation in the Western Hemisphere. p. 33-44. In: H. Schmutterer and K. R. S. Ascher, (eds.), Natural Pesticides From the Neem Tree, GTZ Press, Eschborn, FRG.
- Jones, P. S., S. V. Ley, E. D. Morgan, and D. Santafianos, 1989. The chemistry of the neem tree. p. 19-45. In: M. Jacobson (ed.), Focus on Phytochemical Pesticides, Vol. I (The Neem Tree), CRC Press, Boca Raton, FL.
- Kigodi, P. G. K., G. Blasko, Y., Thebtaranonth, and others, 1989. Spectroscopic and biological investigation of nimbolide and 28-oxonimbolide from Azadirachta indica. *J. Nat. Prod.* 52:1246-1251.
- Klocke, J. A., and I. Kubo. 1982. Citrus limonoid byproducts as insect control agents. *Entomol. Exp. Appl.* 32:299-301.



- Knodel-Montz, J. J., H. G. Larew, and R. E. Webb, 1985. Efficacy of Margosan-O, a formulation of neem, against Liriomyza trifolii (Burgess) on floral crops. USDA Informal Conf. on Liriomyza Leafminers, Beltsville, MD, June 1985, p. 33-44.
- Knodel, J. J., H. G. Larew, and R. E. Webb, 1988. Margosan-O, a commercial formulation of neem seed extract, controls Liriomyza trifolii on chrysanthemums. J. Agric. Entomol. 3:249-254.
- Kubo, I. and J. A. Klocke, 1982. Azadirachtin, insect ecdysis inhibitor. Agric. Biol. Chem. 46:1951-1953.
- Kubo, I., T. Matsumoto, A. Matsumoto, and J. H. Shoolery, 1984. Structure of deacetylazadirachtinol. Application to  $2D^1H$  and  $1H-^{13}C$  shift correlation spectroscopy. Tetrahedron Lett. 25:4729-4732.
- Kubo, I., A. Matsumoto, T. Matsumoto, and J. A. Klocke, 1986. New insect ecdysis inhibitory limonoid deacetylazadirachtinol isolated from Azadirachta indica (Meliaceae) oil. Tetrahedron 42:489-496.
- Ladd, T. L., Jr., M. Jacobson, and C. R. Buriff, 1978. Japanese beetles: extracts from neem tree seeds as feeding deterrents. J. Econ. Entomol. 71:810-813.
- Ladd, T. L., Jr., M. Jacobson, and E. C. Uebel, 1979. Neem tree seed extracts repel Japanese beetles. USDA Agric. Res. 27(9):8-10.
- Ladd, T. L., Jr., 1981. Neem seed extracts as feeding deterrents for the Japanese beetle, Popillia japonica. p. 149-156. In: H. Schmutterer, K. R. S. Ascher, and H. Rembold (eds.). Natural Pesticides From the Neem Tree (Azadirachta indica A. Juss.), GTZ Press, Eschborn, FRG.
- Ladd, T. L., Jr., J. D. Warthen, Jr., and H. G. Klein, 1984. Japanese beetle (Coleoptera:Scarabaeidae): the effects of azadirachtin on the growth and development of the immature forms. J. Econ. Entomol. 77:903-905.
- Larew, H. G., R. E. Webb, and J. D. Warthen, Jr., 1984. Leafminer controlled on chrysanthemums by neem seed extract applied to potting soil. p. 108-117. In: Proc. 4th Ann. Ind. Conf. on Leafminer, Sarasota, FL, Jan. 16-18.
- Larew, H. G., J. J. Knodel-Montz, and R. E. Webb, 1985. The efficacy of neem seed products for the control of Liriomyza trifolii (Burgess) (Diptera: Agromyzidae), p. 34-42. In: proc. 1st Conf. on Insect and Mite Management on Ornamentals, San Jose, CA, Jan. 14-16.
- Larew, H. G., J. J. Knodel-Montz, and R. E. Webb, 1985. Neem seed extract products control a serpentine leafminer in a commercial greenhouse, p. 29-32. In: Proc. Informal Conf. on Liriomyza Leafminers, USDA, ARS, June 1985.
- Larew, H. G., J. J. Knodel-Montz, R. E. Webb, and J. D. Warthen, Jr., 1985. Liriomyza trifolii (Burgess) (Diptera:Agromyzidae) control on chrysanthemums by neem seed extract applied to soil. J. Econ. Entomol. 78:80-84.
- Larew, H. G., 1987. Use of neem seed kernel extract in a developed country: Liriomyza leafminer as a model case, p. 375-385. In: H. Schmutterer, K. R. S. Ascher, and H. Rembold (eds.). Natural Pesticides From the Neem Tree (Azadirachta indica A. Juss.), GTZ Press, Eschborn, FRG.
- Larew, H. G., J. J. Knodel-Montz, and D. F. Marion, 1987. Use of foliar-applied neem (Azadirachta indica A. Juss.) seed extract for the control of the birch leafminer, Fenusa pusilla (Lepelletier) (Hymenoptera: Tenthredinidae). J. Environ. Hort. 5:17-19.

- Larew, H. G., 1988. Limited occurrence of foliar-, root-, and seed-applied neem seed extract toxin in untreated plants. *J. Econ. Entomol.* 81:593-598.
- Lee, S. M., J. A. Klocke, and R. B. Yamasaki, 1986. Isolation and purification of azadirachtin from neem (*Azadirachta indica*) seeds. Abstr. 192nd Natl. Mtg., American Chemical Society, Agrochemistry Div., Anaheim, CA.
- Lee, S. M., J. I. Olsen, M. P. Schweizer, and J. A. Klocke, 1986. Deacetyl-17-hydroxyazadiradione, a new limonoid insect growth inhibitor from *Azadirachta indica*. *Phytochemistry* 27:2773-2775.
- Lidert, Z., D. A. H. Taylor, and M. Thirugnanan, 1985. Insect antifeedant activity of four prieurianin-type limonoids. *J. Nat. Prod.* 48:843-845.
- Lindquist, R. K., H. H. Krueger, and M. L. Casey, unpublished results, 1983.
- Ludlum, C. T., and K. P. Sieber, 1988. Effects of azadirachtin on oogenesis in *Aedes aegypti*. *Physiol. Entomol.* 13:177-184.
- Meisner, J., K. R. S. Ascher, H. Aly, and J. D. Warthen, Jr., 1981. Response of *Spodoptera littoralis* (Boisd.) and *Earias insulana* (Boisd.) larvae to azadirachtin and salannin. *Phytoparasitica* 9:27-32.
- Mikolajczak, K. L., and D. K. Reed, 1987. Extractives of the seeds of the Meliaceae: effects on *Spodoptera frugiperda* (J. E. Smith), *Acalymma vittatum* (F.), and *Artemia salina* Leach. *J. Chem. Ecol.* 13:99-11.
- Mikolajczak, K. L., B. W. Zilkowski, and R. J. Bartelt, 1989. Effect of meliaceous seed extracts on growth and survival of *Spodoptera frugiperda* (J. E. Smith). *J. Chem. Ecol.* 15:121-128.
- Moab, W. J., and J. T. Trumble, 1987. Toxicity, joint action, and mean time of mortality of Dipel 2X, avermectin P<sub>1</sub>, neem and thuringiensin against beet armyworm (Lepidoptera:Noctuidae). *J. Econ. Entomol.* 80:588-592.
- Nakanishi, K., 1975. Structure of the insect antifeedant azadirachtin, p. 283-298. In: C. Runeckles (ed.), *Recent Advances in Phytochemistry*, Plenum, New York, NY, vol. 9.
- Pener, M. P., and U. Shalom, 1987. Endocrine manipulations, juvenile hormone, and ontogenesis of male sexual behavior in locusts. *Insect Biochem.* 17:1109-1113.
- Pener, M. P., D. B. Rountree, S. T. Bishoff, and L. I. Gilbert, 1988. Azadirachtin maintains prothoracic gland titres in *Manduca sexta* pupae: in vivo and in vitro studies, p. 41-54. In: P. Sehnal, A. Zubka, and D. T. Denlinger (eds.), *Endocrinological Frontiers in Physiological Insect Ecology*, Wroclaw Technical University Press, Wroclaw, Poland.
- Pettit, G. R., D. H. R. Barton, D. L. Herald, and others, 1983. Evaluation of limonoids against the murine P388 lymphocytic leukemia cell line. *J. Nat. Prod.* 46:379-390.
- Pliske, T. E., 1984. The establishment of neem plantations in the American tropics. p. 521-525. In: H. Schmutterer and K. R. S. Ascher, (eds.), *Natural Pesticides From the Neem Tree*, GTZ Press, Eschborn, FRG.
- Prabhaker, N., D. L. Coudriet, A. N. Kishaba, and D. E. Meyerdirk, 1986. Laboratory evaluation of neem-seed extract against larvae of the cabbage looper and beet armyworm (Lepidoptera:Noctuidae). *J. Econ. Entomol.* 79:39-41.
- Rajab, M. S., M. D. Bentley, and R. C. Fort, Jr., 1988. Biomimetic formation of a nimbin class limonoid. *J. Nat. Prod.* 51:1291-1293.

Redfern, R. E., J. D. Warthen, Jr., E. C. Uebel, and G. D. Mills, Jr., 1981. The antifeedant and growth-disrupting effects of azadirachtin on *Spodoptera frugiperda* and *Oncopeltus fasciatus*, p. 129-135. In: H. Schmutterer, K. R. S. Ascher, and H. Rembold (eds.). Natural Pesticides From the Neem Tree (*Azadirachta indica* A. Juss.), GTZ Press, Eschborn, FRG.

Redfern, R. E., T. J. Kelly, A. B. Borkovec, and D. K. Hayes, 1982. Ecdysteroid titers and molting aberrations in last-stage *Oncopeltus* nymphs with insect growth regulators. *Pesticide Biochem. Physiol.* 18:351-356.

Redfern, R. E., J. D. Warthen, Jr., M. Jacobson, and J. B. Stokes, 1984. Antifeeding potency of neem formulations. *J. Environ. Sci. Health.* A19:477-481.

Reed, D. K., and G. L. Reed, 1981. Natural products repel cucumber beetle. *Agric. Res. (USDA)* 30(2):12.

Reed, D. K., M. Jacobson, J. D. Warthen, Jr., and others, 1982. Cucumber beetle antifeedants: laboratory screening of natural products. *USDA, Sci. Educ. Admin., Tech. Bull.* 1641.

Reed, D. K., J. D. Warthen, Jr., E. C. Uebel, and G. L. Reed, 1982. Effects of two triterpenoids from neem on feeding by cucumber beetles (Coleoptera:Chrysomelidae). *J. Econ. Entomol.* 75:1109-1113.

Schroeder, D. R., and K. Nakanishi, 1987. A simplified isolation procedure for azadirachtin. *J. Nat. Prod.* 50:241-244.

Stein, U., 1984. The potential of neem for inclusion in a pest management program for control of *Liriomyza trifolii* on chrysanthemum. M.S. thesis, University of California, Riverside, 86 pp.

Thompson, E. B., and C. C. Anderson, 1978. Cardiovascular effects of *Azadirachta indica* extract. *J. Pharm. Sci.* 67:1476-1478.

Uebel, E. C., J. D. Warthen, Jr., and M. Jacobson, 1979. Preparative reversed-phase chromatographic isolation of azadirachtin from neem kernels. *J. Liquid Chromatog.* 2:875-882.

Warthen, J. D., Jr., R. E. Redfern, E. C. Uebel, and G. D. Mills, Jr., 1978. An antifeedant for fall armyworm larvae from neem seeds. *USDA Sci. Educ. Admin., Agric. Res. Results* ARS-NE-1, 1-9.

Warthen, J. D., Jr., E. C. Uebel, S. R. Dutky, and others, 1978. Adult house fly feeding deterrent from neem seeds. *USDA Sci. Educ. Admin., Agric. Res. Results* ARS-NE-2, 11 p.

Warthen, J. D. Jr., and E. C. Uebel, 1981. Effect of azadirachtin on house crickets, *Acheta domesticus*, p. 137-147. In: H. Schmutterer, K. R. S. Ascher, and H. Rembold (eds.). Natural Pesticides From the Neem Tree (*Azadirachta indica* A. Juss.), GTZ Press, Eschborn, FRG.

Warthen, J. D., Jr., J. B. Stokes, M. Jacobson, and M. P. Kozempel, 1984. Estimation of azadirachtin content in neem extract and formulations. *J. Liquid Chromatog.* 7:591-598.

Webb, R. E., M. A. Hinebaugh, R. K. Lindquist, and M. Jacobson, 1983. Evaluation of aqueous solution of neem seed extract against *Liriomyza sativae* and *L. trifolii* (Diptera:Agromyzidae). *J. Econ. Entomol.* 76:357-362.

Weidhaas, D. E., unpublished results, 1978.

Yamasaki, R. B., and J. A. Klocke, 1987. Structure-activity relationships of azadirachtin, a potential insect control agent. J. Agric. Food Chem. 35:467-471.

Yamasaki, R. B., and J. A. Klocke, 1989. Structure-bioactivity relationships of salannin as an antifeedant against the Colorado potato beetle (Leptinotarsa decemlineata). J. Agric. Food Chem. 37:1118-1124.

Zanno, P. R., 1974. The structure of azadirachtin, a potent insect phagorepellent and systemic growth disruptor from Azadirachta indica. Ph.D. thesis, Columbia University, New York, NY, 46 pp.

Zehnder, G., and J. D. Warthen, Jr., 1988. Feeding inhibition and mortality effects of neem-seed extract on the Colorado potato beetle (Coleoptera:Chrysomelidae). J. Econ. Entomol. 81:1040-1044.

H. Schmutterer

## ABSTRACT

Despite a considerable, worldwide increase of research on neem during the last decade, many problems remain to be solved. Some of them refer to the cultivation of the neem tree, aiming at high fruit yields and high contents of azadirachtin. After clarification of the environmental and genetic factors involved intensive breeding work could lead to new ways for the economic production of raw material with high quality for extracts of various kinds. The development of new techniques for harvesting, depulping, drying and storage of neem seeds is also desirable.

Concerning simple but laborious water and solvent extracts, there are problems of acceptance in developing countries. They should be overcome by the help of socio-economic studies at least in such regions where farmers poor in resources have no alternatives to home-made botanicals.

Step by step worldwide research work should widen the spectrum of neem-sensitive agricultural pests. Some pest groups have been neglected in the past in trials with neem products, specially those with piercing-sucking mouthparts, except plant- and leafhoppers. Some of them are difficult to control, for instance thrips and some aphid species. In some cases research on new formulations may help to solve the present problems.

More research work should be devoted to the sterilizing and fitness-reducing effects of neem on adult insect pests to develop new methods of environmentally sound control. The phase-modifying efficacy of neem oil after contact with nymphs of locust species is of particular interest for African countries. There is also a wide open field for research aiming at the integration of neem products in integrated pest management (IPM) systems which will be an interesting but cumbersome task in developing and industrialized countries with increasing environmental and resistance problems. Selective pesticides like neem are badly needed for IPM in agriculture and elsewhere.

## INTRODUCTION

Steadily growing problems of pest resistance to synthetic pesticides, pollution of the environment including groundwater and air, as well as side-effects on natural enemies and other organisms necessitate a worldwide intensive search for new pesticides that exhibit such negative properties only to a lesser extent or better not at all. Many research workers hope that the kingdom of plants, after a long coevolution with phytophagous organisms, could be a promising source for such compounds. In fact, plants contain a huge number of chemicals, acute toxic ones and others, which are active against many insects and other animals.

The search for plant ingredients with insecticidal, nematocidal and fungicidal properties was intensified during the last decade and has led to a number of promising results. Presently, the neem tree, Azadirachta indica, and the main active principle from its seed kernels, the tetranortriterpenoid azadirachtin, seem to occupy an outstanding position. This can be explained in part by the effectiveness of azadirachtin against many insect pests, the very good chances for integration of the neem tree on a worldwide scale in semi-arid environments and the numerous uses its products provide. The unique properties of its active principle(s) have also attracted many research workers.



## NEEM TREE RESEARCH

In this paper, which can never be comprehensive, it is tried to concentrate on a number of topics of special interest in future neem research in relation to agricultural needs in developing and industrialized countries. A separation of these two groups of countries is not appropriate as most problems are common, others are only on different levels of technology.

The first topic is the neem tree itself as the source of the pesticide at least as long as an economic synthesis of neem derivatives is not realistic. We urgently need more informations on factors influencing fruit yield and that of active ingredient(s). To satisfy the future demand, many more neem trees have to be planted, preferably in semi-arid regions of the tropics and subtropics. For successful pest control about 30-50 g of azadirachtin are needed per hectare which is little compared to many synthetic compounds. However, about 10 kg of seed kernels or more are needed in most countries to achieve this level of control. At present there are practically no reliable informations on how to plant which ecotypes in which regions for an optimum fruit yield and azadirachtin production. The azadirachtin contents measured by our research group in seed kernel extracts were 1,1 to 6,8 mg/g (Ermel, pers. commun.). Most probably soil conditions, rainfall (optimum perhaps 800-1200 mm per year), availability of ground water during long dry seasons, temperature (altitude) and genetic factors should be important. If plantations are established, the intraspecific competition for light, water and nutrients could be significant.

Which problems may develop in practice is demonstrated by some examples from the Caribbean. In the western parts of the island of Hispaniola, in Haiti, many neem trees were planted about ten to six years ago by US-AID, mainly to provide firewood for this nearly completely deforested country (Pliske, 1983). The seeds came from West Africa (Nigeria). Nowadays, hundreds of thousands of neem trees have grown up and yield thousands of tons of fruit but the kernels have only a relatively low content of azadirachtin (Ermel, pers. commun.). This does not mean that they are unsuitable for home-made water extracts, oil production etc. but their attractiveness for export is lower compared to seeds from parts of West Africa with a high azadirachtin content. Nobody knows whether this low content is caused by environmental or genetic factors or both. Interestingly, the azadirachtin content of a Togolese progeny, planted in a high rainfall area (1800-2000 mm/year) in the neighbouring Dominican Republic, is also lower than in Togo itself. This leads to the assumption that environmental factors play an important role, which will be underlined by a third example: In the western parts of Ecuador, several thousand neem trees, derived from Nigerian seeds planted about nine years ago, produce at least every second year top yields of fruit with high azadirachtin content. In this region the annual rainfall amounts to about 800-1000 mm and there is a long dry season, during which, as a rule, groundwater is available for the trees.

Urgently needed further research on the neem tree itself should aim at characterizing the best ecotypes for defined environmental conditions. If this is realized, suitable material should be available for breeding and selection work to obtain good yields of fruit and active ingredient(s) at the same time.

## QUALITY OF FRUIT AND METHODS OF EXTRACTION OF ACTIVE PRINCIPLE(S)

Additional research work is required to improve harvesting of fruit, depulping, drying and storage. Some West African countries are in a lucky situation as fruit bats and birds depulp the seeds and spit them out. Nevertheless, collectors of neem seeds have to be informed worldwide how to treat them to get a good quality by avoiding mechanical damage and fungal infection.

The time-consuming work of collecting, depulping and drying of considerable amounts of neem seeds is one of the main reasons for lack of acceptance of home-made neem pesticides, for instance water extracts, seed powder, seed cake and oil. Ready-to-use neem pesticides, based on alcoholic extracts and sold in bottles, have better chances if the consumers have financial resources, for instance vegetable growers. However, this should not prevent governmental or private foreign aid organizations to propagate home-made neem pesticides for small farmers to provide a change for them to solve some of their pest problems practically without costs. Socio-economic research

is needed to clarify the real reasons for non-acceptance problems of simple neem products among small farmers.

Methods for alcoholic extraction of active ingredient(s) from neem seed kernels, first published by Feuerhake and Schmutterer (1982), were improved during recent years by activities of various chemical firms in different parts of the world (Larson, 1987). Other firms utilized the property of water to extract azadirachtin selectively (Kleeberg, 1990). This is of great importance for the economic production of marketable neem pesticides, specially in industrialized countries. The same applies to formulations. The shelf-life of azadirachtin in formulated products, specially under extreme tropical conditions (India etc.), is still a problem. Further progress by intensive research in this field is desirable, also including other active ingredients than azadirachtin in neem extracts. This could also improve the chances of progenies with low azadirachtin contents (Haiti, Niger, Sudan). Synergists could be used for formulations for industrialized countries. Further simple methods for production and application of neem oil, neem seed powder, neem seed cake and seed kernel water extracts should be developed for developing countries; they may improve the acceptance of such products.

#### MODE OF ACTION

Research work during the last two decades has led to the final elucidation of the main active principle azadirachtin and to considerable progress regarding the understanding of its mode of action. The hypothesis of Barnby and Klocke (1990), e.g. the reduction of the molting hormone titers by reducing prothoracicotropic hormone (PTTH) titers and the ability of prothoracic glands to produce ecdysone via stimulation by PTTH is rather convincing. Other research on neem's mode of action pointed in the same direction (Sieber and Rembold, 1983). This means that azadirachtin is an antihormonally active substance in a broad sense without being a true antihormone itself. In spite of these results further research on neem's physiological mode(s) of action remains desirable.

The variety of neem effects on insects is of particular interest both for basic and applied research. There are repellent, antifeedant, antiovipositional, growth regulating (IGR), sterilizing and fitness-reducing effects which vary from one insect species to another. In case of a synthesis of parts of the azadirachtin molecule, which has been tried by various working groups (Ley et al., 1987), all effects of the natural compound should be preserved if possible. Otherwise the performance under field conditions might be unsatisfactory. The "primary" antifeedant effect (Schmutterer, 1988) is not enough as it plays only a subordinate role in many species.

#### SPECTRUM OF TARGET INSECTS

The spectrum of insect pests sensitive to neem was partly clarified during the last ten years (Warthen, 1989; Saxena, 1989). In the beginning of this research work it was suggested that only a few insect species can be controlled by using neem products. This opinion has changed completely in the meantime as many pests proved to be sensitive in laboratory and field experiments. Therefore neem products can be called medium- to broad-spectrum pesticides. Neem also showed good effects against pests that became resistant to synthetic compounds, for example *Plutella xylostella*, *Scrobipalpus absoluta*, *Liriomyza trifolii* and *Bemisia tabaci*. Table 1 and 2 demonstrate the effect of neem pesticides in field trials in South America (Ecuador) and in the Caribbean (Dominican Republic), respectively. Water extracts of seeds, oil and seed powder were applied which means products that can be produced by farmers themselves.

The first example (Table 1) from western Ecuador stems from a region where corn, rice and peanuts are grown as the main crops. It demonstrates that water extracts and seed powder (funnel treatment of corn against *Spodoptera frugiperda*) can control all major pests of the three crops with the same degree of efficacy as the standard insecticide permethrin (Ambush). The number of applications was also identical. The main pests were *S. frugiperda* and *Heliothis virescens* on corn, *Mocis latipes* and *Oebalus ornatus* on rice and *Anticarsia gemmatilis* and *Stegasta bosquella* on peanut.

Table 1

Effect of neem products and permethrin on major pests of main field crops in field trials 1987-89 in western Ecuador (compiled after Wendt, unpubl. data).

Crop	Pests	Neem products	Conc.	Treated Instars	Efficacy	Number of applications
Corn	<u>Spodoptera frugiperda</u>	NSP <sup>1</sup>	2g/fun- nel	larvae	++	1
	<u>Helicoverpa zea</u>	NSWE <sup>2</sup>	50g/l	"	+	1
	<u>Mocis latipes</u>	"	"	"	++	1-2
Rice	<u>Oebalus ornatus</u>	"	"	adults	-	2
	<u>Stegasta bosquella</u>	"	"	larvae	+	2-3
Peanut	<u>Anticarsia gemmatilis</u>	"	"	"	++	1

1 = Neem seed powder mixed with sawdust (1:1,v/v); application by hand.

2 = Neem seed water extract.

+ = Good control, comparable to permethrin (75-150 ml Ambush/ha).

++ = Very good control, comparable to permethrin but effect 3-4 d delayed. Number of applications of neem products and permethrin identical. Azadirachtin content of neem seed kernels used in the trials: ca. 3-4 mg/g.

- = No obvious effect.

The second example (Table 2) refers to tomato whose pests and their control with neem products were studied from 1987-90 in the Dominican Republic. The main pests were Bemisia tabaci (since 1988), Keiferia lycopersicella, Liriomyza trifolii, Helicoverpa zea, Heliothis virescens and Spodoptera spp. Altogether about twenty insect pest species were identified on this crop.

All major pests were controlled by neem applications to a larger or lesser extent. Adult flea beetles (Eplatrix sp.) and adult bugs were less or not sensitive which was not surprising as neem mainly acts as a growth regulator. For the control of L. trifolii higher concentrations (ca. 70 g of seeds per liter of water) were needed in severe outbreaks, compared to 50-60 g/l against most other pests. B. tabaci, which showed a heavy outbreak in 1988/89 season, was sensitive to water extracts and oil. However, higher concentrations of water extracts (> 60 g seeds per liter) and specially oil (> 1 %/l of water) led to phytotoxic effects and reduction of yields despite good effects on pests. The same applied to a product based on soap. The best results were obtained in trials in which neem products and synthetic pesticides like deltamethrin were sprayed by turns.

Based on the results of worldwide field trials it can be concluded that neem insecticides control nymphs of grasshoppers and locusts, termites (cake), bugs, leaf- and planthoppers, some aphid species (after repeated application of water extracts) and whiteflies (specially oil), larvae of sawflies, butterflies and moths, beetles and flies. Most thrips, mealybugs and armored scales as well as some other groups of insects are not sensitive or only to a low degree. Sterilizing effects were observed in adults of Heteroptera, Homoptera and Coleoptera (phytophagous Coccinellidae and Chrysomelidae) (Steets and Schmutterer, 1975; Schmutterer, 1987; Dorn et al., 1987).

#### FITNESS-REDUCING EFFECTS

Fitness-reducing effects of neem products on adult insects could be of special interest for future pest control. They result, for example, in the loss or reduction of flying ability (Wilps, 1989), impotence of males (Dorn et al., 1987), inability to recognize pheromones (Steffens and Schmutterer, 1982) etc. Strong contact effects of neem oil have been recorded in locusts, leading to transformation of gregarious nymphs to



Table 2

Effect of neem products on tomato pests in the Dominican Republic in field trials 1987-90 (compiled after Serra, unpubl. data).

Pest	Neem product	Conc.	Treated instars	Efficacy	Remarks
Prostigmata					
<u>Aculops ly-</u> <u>copersici</u>	NO	2.5-3%	all	++	phyto- toxicity
Heteroptera					
<u>Nezara</u> <u>virdula</u>	NSWE	20-90g/l	nymphs, adults	+ - ++	effect on nymphs,
<u>Phthia picta</u>	"	"	"	"	no obvious
<u>Enschistus</u> <u>bifibulus</u>	"	"	"	"	effect on adults
Homoptera					
<u>Bemisia</u> <u>tabaci</u>	NO+emulg. NSWE	0.75-3% 40-50g/l	all	+++ ++	NO conc.>1% phytotoxic
Coleoptera					
<u>Epitrix</u> <u>fuscata</u>	NSWE	20-90g/l	adults	-	sterilization?
<u>Diabrotica</u> <u>balteata</u>	"	"	"	+	mortality
Lepidoptera					
<u>Kelferia ly-</u> <u>copersicella</u>	NO NSWE	2.5-3% 20-90g/l	larvae	++ ++-+++	NO phytotox.
<u>Herpetogramma</u> <u>bipunctalis</u>	NSWE	"	"	+	low suscep- tibility
<u>Manduca</u> <u>sexta</u>	"	"	"	+++	high sus- ceptibility
<u>Heliothis</u> <u>virescens</u>	NO NSWE	2.5-3% 20-90g/l	"	++ +-+++	-
<u>Helicoverpa</u> <u>zea</u>		"	"	"	-
<u>Spodoptera</u> <u>latifascia</u>		"	"	"	-
<u>Spodoptera</u> <u>ornithogalli</u>		"	"	"	-
<u>Trichoplusia</u> <u>ni</u>	NSWE	20-90g/l	"	+ - +++	-
<u>Pseudoplusia</u> <u>includens</u>	"	"	"	"	-
Diptera					
<u>Liriomyza</u> <u>trifolii</u>	NO NSWE	2.5-3% 20-90g/l	"	+++ +-+++	-

NO = Neem oil.

NSWE = Neem seed water extract. Azadirachtin content of neem seed kernels used in the trials: ca. 3-3.5 mg/g.

+ = Fair control (~60% mortality), ++ = good control (~70-80% mort.),  
+++ = very good control (> 90% mort.).

intermediate and solitary forms, for instance in Schistocerca gregaria, combined with a complete change of behavior (Freres, 1989; Schmutterer and Freres, 1990) (Figure 1). Sterilizing and fitness-reducing effects, including change of phases in locusts, should be special topics of future neem research as they may open new ways for integrated pest management.

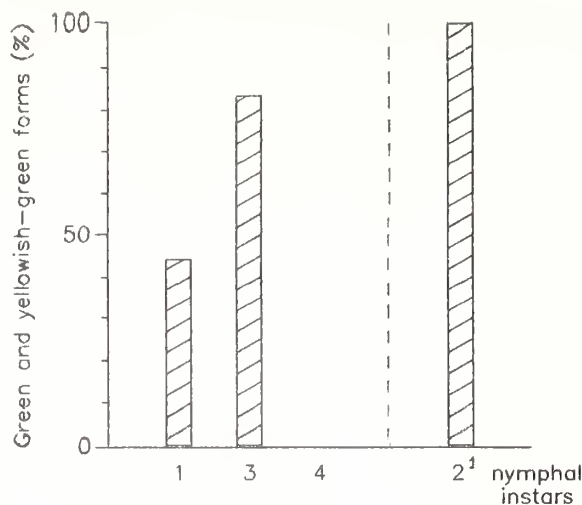


Figure 1. Percentage of green or yellowish-green (solitary and intermediate) forms of *Schistocerca gregaria* after application of neem oil (NO; 1ml/m<sup>2</sup>) on gregarious nymphs and their foodplants (compiled after Freres, 1989). Azadirachtin content of NO: 0.015%.

2<sup>1</sup> Application of NO, enriched with 1 % alcoholic neem seed kernel extract (AZT-VR-K; azad. cont. 1.5%).

#### PHYTOTOXICITY

It can be concluded from the results of the above described experiments on tomato that research work on phytotoxicity of neem is desirable. It was also observed on onion, potato and cabbage (Kirsch, 1987; Zehnder und Warthen, 1988; Freisewinkel, 1989). In the latter crop, for instance, the heads of white cabbage become smaller than untreated heads, provided the pests are kept away from the latter. In this vegetable and in onion the waxy outer layer of the leaves is destroyed by neem oil which may lead to increased transpiration. Probably phytotoxic effects can be reduced by lower concentrations or special formulations of neem products.

#### NEEM AND IPM

The development of IPM-conform concepts including neem is of special importance. The combination of neem applications with other IPM elements, including biological control, should be studied with emphasis to allow the successful control of pest complexes, as well as the integration with selective pesticides. Examples from Australia of combinations of neem application with lures offered in traps are of special interest within this context (Rice, pers. commun.).

To practice IPM concepts with special consideration of natural enemies of pests future research work should aim at obtaining further results on non-existing or slight side-effects of neem products on parasitoids and predators. The work of Lamb and Saxena (1988) on very slight side-effects on enemies of rice leaf rollers in the Philippines is a convincing example. When doing such work, a clear differentiation between laboratory and field experiments should be made. Kaethner (pers. commun.) found that neem products, such as oil, had a strong growth regulating effect on larvae of *Chrysoperla carnea* and *Coccinella septempunctata* in the laboratory on glass-sheets but not on plants in the field.

#### CONCLUSIONS

To sum it up it can be said that alternative, environmentally sound pesticides including botanicals are badly needed in agriculture on a worldwide scale. They have to be considered in IPM concepts and may help to overcome increasing problems with

resistant insects. Exclusive neem spraying regimes are not recommended to avoid or postpone an adaptation of pests. Planting of huge numbers of promising ecotypes of the neem tree in suitable environments is essential to meet the future demand for raw material, regardless whether simple or more sophisticated neem products are applied in developing or industrialized countries. There is a wide field for research work on neem, specially for further studies on sterilizing and fitness-reducing properties of the active ingredient(s). They may yield new IPM-conform concepts, based on the unique mode(s) of action of this remarkable botanical.

## REFERENCES

- Barnby, M.A. and J.A. Klocke. 1990. Effects of azadirachtin on levels of ecdysteroids and prothoracicotropic hormone-like activity in Heliothis virescens (Fabr.) larvae. J. Insect Physiol. 36:125-131.
- Dorn, A., Rademacher, J.M., and E. Sehn. 1987. Effects of azadirachtin on reproductive organs and fertility in the large milkweed bug, Oncopeltus fasciatus. Proc. 3rd Int. Neem Conf. Nairobi 1986, GTZ Eschborn (FRG), 273-288.
- Ermel, K., pers. commun.
- Feuerhake, K. and H. Schmutterer. 1982. Einfache Verfahren zur Gewinnung und Formulierung von Niem samenextrakten und deren Wirkung auf verschiedene Schadinsekten. Z. PflKrankh. PflSchutz 89:737-747.
- Freisewinkel, D. 1989. Freilanduntersuchungen zur Wirkung von Niem samenextrakten gegen Schädlinge in Zwiebelkulturen in der Dominikanischen Republik. Diploma thesis, University of Giessen, FRG.
- Freres, T. 1989. Die Wirkung von Niemöl auf Wanderheuschrecken. Diploma thesis, University of Giessen, FRG.
- Kaethner, M., pers. commun.
- Kirsch, K. 1987. Studies on the efficacy of neem extracts in controlling major pests in tobacco and cabbage. Proc. 3rd Int. Neem Conf. Nairobi 1986, GTZ Eschborn (FRG), 495-515.
- Kleeberg, H. 1990. Das NiemAzal-Konzept (IV). Entwicklung eines botanischen Insektizids. Trifolio GmbH, Lahnau (FRG).
- Lamb, R. and R.C. Saxena. 1988. Effects of neem seed derivatives on rice leaffolders (Lepidoptera: Pyralidae) and their natural enemies. Final Worksh. IRRI-ADB-EWC Proj. Bot. Pest Contr. Rice-Based Cropp. Syst., Dec. 1988, Los Baños, Philipp.
- Larson, R.O. 1987. Development of Margosan-O, a pesticide from neem seed. Proc. 3rd Int. Neem Conf. Nairobi 1986, GTZ Eschborn (FRG), 243-250.
- Ley, S.V., Santafianos, D., Blaney, W.M., and M.S.J. Simmonds. 1987. Synthesis of a hydroxydihydrofuranacetal related to azadirachtin: a potent insect antifeedant. Tetrahedron Lett. 28:221.
- Pliske, T.E. 1984. The establishment of neem plantations in the American tropics. Proc. 2nd Int. Neem Conf. Rauischholzhausen 1983, GTZ Eschborn (FRG), 521-525.
- Rice, M., pers. commun.
- Saxena, R.C. 1989. Insecticides from Neem. Insecticides of Plant Origin, ACS Symp. Ser. 387, Amer. Chem. Soc. Washington D.C., 110-135.
- Schmutterer, H. 1987. Fecundity-reducing and sterilizing effects of neem seed kernel extracts in the Colorado potato beetle, Leptinotarsa decemlineata. Proc. 3rd Int. Neem Conf. Nairobi 1986, GTZ Eschborn (FRG), 351-360.
- Schmutterer, H. 1988. Potential of azadirachtin-containing pesticides for integrated pest control in developing and industrialized countries. J. Insect Physiol. 34:713-719.

Schmutterer, H. and T. Freres. 1990. Influence of neem-seed oil on metamorphosis, colour and behaviour of the desert locust, Schistocerca gregaria (Forsk.), and of the African migratory locust, Locusta migratoria migratorioides (R. & F.). Z. PflKrankh. PflSchutz, in print.

Serra, C., pers. commun.

Sieber, K.-P. and H. Rembold. 1983. The effects of azadirachtin on the endocrine control of moulting in Locusta migratoria. J. Insect Physiol. 29:523-527.

Steets, R. and H. Schmutterer. 1975. Der Einfluß von Azadirachtin auf die Lebensdauer und das Reproduktionsvermögen von Epilachna varivestis Muls. (Coleoptera: Coccinellidae). Z. Pfl.Krankh. PflSchutz 82:176-179.

Steffens, R. and H. Schmutterer. 1982. The effect of a crude methanolic neem (Azadirachta indica) seed kernel extract on metamorphosis and quality of the Mediterranean fruitfly, Ceratitis capitata Wied. (Diptera, Tephritidae). Z. ang. Ent. 94: 98-103.

Warthen, J.D.Jr. 1989. Neem (Azadirachta indica A. Juss): Organisms affected and reference list update. Proc. Entomol. Soc. Wash. 91: 367-388.

Wendt, U., pers. commun.

Wilps, H. 1989. The influence of neem seed kernel extracts (NSKE) of the neem tree Azadirachta indica on flight activity, food ingestion, reproduction rate and carbohydrate metabolism in the Diptera Phormia terraenovae (Diptera, Muscidae). Zool. Jb. Physiol. 93: 271-282.

Zehnder, G. and J.D. Warthen. 1988. Feeding inhibition and mortality of neem-seed extract on the Colorado potato beetle (Coleoptera: Chrysomelidae). J. Econ. Entomol. 81: 1040-1044.

## COMMERCIALIZATION OF NEEM

### 245 COMMERCIALIZATION OF THE NEEM EXTRACT MARGOSAN-O IN A USDA COLLABORATION

Robert O. Larson

The neem tree (Azadirachta indica A. Juss.) a tropical evergreen of the family Meliaceae offers a cornucopia of gifts to mankind through the effective use of many parts of the tree. The USDA-Beltsville, MD in a unique collaboration with Vikwood Ltd. of Sheboygan, Wisconsin, USA developed a botanical pesticide of unusual effectiveness against a wide spectrum of insect pests through a variety of actions. The molecule, Azadirachtin, a tetranortriterpenoid is extracted from the neem seed and incorporated into a harmonious host solution which, to date, has shown up to 24 months shelf-stability and has shown no resistance build-up in any of the test insects. EPA registration was achieved for Margosan-OR on non-food crops and trees after successful completion of a battery of specially tailored toxicity studies for this product. The unique formulation and process has been granted U. S. Patent No. 4,556,562 and was recently sold to W. R. Grace & Co., Cambridge, MA 02140.

The full story of Margosan-0, a new pesticide made from an extract of the seed of the neem tree began over 4,000 years ago in India when the first word of the neem tree appeared in Sanskrit writings of the Vedic period. The neem tree, Azadirachta indica, which means "Free tree of India", in Persian, is a member of the Meliaceae family, a mahogany species. But, wait...those of you invited here have the combined distillation of accumulated knowledge of neem around the world, and you all know about the neem tree.

I thought, therefore, that I might spend some time answering one question asked of me so many times since 1979. "How and why did you get into the neem program, Robert?"

It all started with this little clipping which I read in the Atlanta Journal one day between planes at the airport there. It read in part...  
"...Experiments by the U. S. Department of Agriculture scientists indicate that Japanese beetles, a major crop pest, would rather starve than eat plants treated with tiny amounts of extract of the neem tree. Martin Jacobson, USDA research chemist said in experiments, fantastically low doses of a neem seed extract called azadirachtin protected plant leaves"... and so forth.

The neem tree? A flood of memories rushed over me and I was transported back to 1973 when I visited Nagpur in central India. I remember that tree. I was in Teddy Sonti's front yard on the patio one night and the bright blue-white fluorescent bulbs lit up the whole yard. The conversation went on like this:

"Teddy, how come that even with these bright fluorescent bulbs lighting up the place, there are no bugs to be seen?"

"I think it has something to do with those two trees in the corner of my compound. Those are neem trees."

"What's a neem tree?"

"It's a Meliaceae, a mahogany variety."

"Okay, but what does it do?"

"They say that when the wind blows through the neem tree it becomes freshened."

"What do you mean freshened?"

"Well, the air takes on some kind of odor that flying insects don't like and they stay upwind of the smell."

---

Larson is President of Vikwood Ltd., 1221A Superior Avenue, Sheboygan, Wisconsin 53081.



So I walked over to a tree and smelled the leaves but detected no particular odor. Then I chewed a leaf and spat vigorously since the leaves were quite bitter. The conversation went on:

"What else can you tell me about the tree?"

"My wife, Pooni, collects the seeds at the time of fruiting and washes and dries them and puts them in with our food grains."

"So what happens?"

"No bugs get into our food bins."

"Really. What else?"

"Well, she takes fresh leaves and puts them under our mattresses and then no bugs crawl up into our beds."

"No kidding. What else?"

"She puts leaves in our books to keep insects from eating the paper or the bindings and she stores our woolens in the summer with neem leaves to keep moths from laying eggs."

"Anything else?"

"Oh, yes. You can crush the leaves and rub them on your exposed body parts and no mosquitoes will bite you."

"Wow! What else?"

"Another thing. You boil the leaves in water, cool it and apply it to your body as a lotion. It cures scabies, prevents scarring from poxes, and generally relieves rashes and things."

"Really. Anything else?"

"The twig is broken off and used as a toothbrush. It reduces cavities and prevents gum disease."

"Fantastic! How many people in India use this every day?"

"I'd say about 75%."

"Wow! You mean to tell me that over 350 million people use the neem twig in India alone, and we don't know about this stuff in the West? Why not?"

"No money in it, I guess."

"Do you use the neem twig on your teeth?"

"No, I use Colgate. But my father used the twig every day and lived to be 84 and never had a cavity in his head."

All this was told to me by Mr. V. R. Sonti, whose father invented the CCA wood treatment with the green tint that you see in all the lumber yards now. Mr. Sonti and his father were for many years the sole source of this product. He then went on to tell me about the medical uses of neem by Ayurvedic doctors in the towns and villages all over India. All of this came back to me while in Atlanta, so I phoned Martin Jacobson and told him what I knew of the tree. He invited me to visit them in Beltsville if I ever got to the Washington area.

Soon I came to the East Coast and visited the USDA-Beltsville. I learned about the progress they were making with a neem extract and met Dr. Waldemar Klassen and the "Neem Team" headed by Martin Jacobson and included Dave Warthen, Jerry Stokes, Hiram Larew and Ralph Webb, to name a few. They asked me what took me to India and I told them something of my wood import program which had its start in India. They asked me if I could help them locate more potent seed, and within a few weeks of my request to contacts in India, I received seed which assayed out at ten times more azadirachtin than seed supplied through the U. S. Embassy. This was purely accidental, however, since Western inquiry was in its infancy.

Shortly thereafter, I was asked if I was willing to collaborate on a joint effort to commercialize a neem product from a seed extract. I asked why they were talking to me when there were so many chemical companies in a much better position to participate. They stated that those companies would not work with a natural product and would try to synthesize the azadirachtin molecule, patent it, and sew up the market. I found this hard to believe, but during the ensuing years it proved to be quite true.

A conference was called by Dr. Klassen and the joint effort was divided as follows. Vikwood Ltd. was to take the extract made by the USDA-Beltsville and seek to improve its stability and devise a more efficient method of extraction. The USDA-Beltsville was to do the testing of the extract on target insects which damaged high value commercial crops in horticulture and agriculture to the extent of billions of dollars annually. The estimate at that time was that 30% of all crops were ruined in the field by marauding insects or eaten in storage due to poor protection methods.

Lacking laboratory and staff, I sought out help in my area and located D & S Associates, a very capable botanical laboratory and consultants to a number of food processors. Together we set out criteria to be met, dictated largely by the goals sought by the USDA Neem Team. I understood these to be:

- a. Efficacy against a broad spectrum of insects during one or more life stages.
- b. Stability after a long period of shelf-life, say at least up to 1 year.
- c. Inexpensive for field use.
- d. Non-toxic to warm-blooded mammals.
- e. Low or no toxicity to non-targeted insects.
- f. Ease of mixing, using a simple water diluent.
- g. High concentration of active ingredient.
- h. Residual activity in the field of 1 month or less.
- i. Assured, safe decomposition into harmless residues.
- j. Ultraviolet stability.
- k. Non-phytotoxic to plant tissue.

After several years of amending the original formulation, we began to understand the azadirachtin molecule better and got improved stability on the shelf and improved performance in the field. By 1983, we had a workable product and submitted it to the EPA for registration on non-food crops and to the U. S. Patent Office for a patent on the method of extraction of azadirachtin and the stabilizing process.

I will not dwell on the many frustrations in dealing with the EPA other than to say it was the darkest period of my life, having to work with an advocative agency like the USDA on one hand and the adversarial EPA on the other; a governmental dichotomy.

The EPA finally confessed in writing that they were having difficulty in registering the product due to the "uniqueness of the formulation and chemical type." Ironically, that letter was used to help me obtain the patent since it stood the test of "Novel Approach", a recent new guideline in American patentability of products and ideas.

The product was named Margosan-O (the O stands for oil) after the margosa tree, the Portuguese name for the neem tree, the source of the first neem seeds I located in Bombay. I had planned to make three products: Margosan with little or no oil for better systemic effect; Margosan-O made with some of the oils for UV protection and better surfactance; and ... Margosan-D, a dust-based product. The extreme difficulties of dealing with the EPA discouraged any further attempt on my part to attempt anything else and the other two projects were abandoned.

By 1985, the USDA had committed over 8 years of effort and some three and a half million dollars on the Neem Program and Vikwood Ltd. tried to introduce Margosan-O to an unbelieving market.

A contract extraction company heard of my efforts and told me that their unique extraction process was ideal for efficient extraction of an azadirachtin-rich product and so we came to the agreement to have Margosan-O made out of state under contract. I was unable to locate venture capital on such a chancy project, but since I had developed Margosan-O without any foundation grant assistance or outside funds, I felt that if the USDA-Beltsville had confidence in the product, I would have to go ahead on my own resources.

Although many samples were sent to USDA testing stations and to private horticultural and agricultural researchers here and abroad, responses were few in number and slow in coming. Nonetheless, the responses that I did get were encouraging and Margosan-O was sold in limited quantities for a year and a half with generally good results and stability. I finally ordered 9,000 pints to be made, the anticipated yield from my stock of Indian seed. The contract company missed the May deadline despite insisting on advance payment, and when they finally got around to produce the product in December, 1986, they found they could not stabilize the final product and I was mired down.

Ultimately, I was introduced to W. R. Grace & Co., which was looking for a viable pesticide of benefit to the environment and soon we had a "fit".

Margosan-O is now a viable product having 3000 ppm azadirachtin in a homogeneous and harmonious solution which contains various stabilizers, buffers and sunscreens, and controls a wide spectrum of pests in one or more of the following ways:

Repellency - Many insects will not approach a plant sprayed with a neem extract.

Antifeedancy - Some insects, such as the desert locust will alight on a sprayed plant but will starve before feeding on the plant.

Growth regulation through hormonal disruption - The activity is not through a gut poison but acts on the programmed metamorphosis of the insect and somehow short-circuits the life stages.

Oviposition deterrence - Many female insects will refuse to lay eggs on sprayed plants.



Egg hatching suppression - The action in this test is not clearly understood but the result was non-hatching of eggs for a period of 4 weeks following limited spraying.

Margosan-0 is non-toxic to mammals, earthworms, good predator insects, lady bugs and honeybees, to name a few. When used as directed, it is non-phytotoxic to plant tissue and can be used as a foliar spray diluted with up to 150 parts water, or in the case of mining insects used as a soil drench when diluted with up to 300 parts water, with good systemic uptake. Margosan-0 has shown no resistance build-up to targeted insects in over 2 years of continuous testing.

The Federal Register battery of tests could not be used to test Margosan-0, due to its high ethanol formulation, so the EPA after lengthy deliberation and discussion, wrote out specially tailored toxicity tests to be run. This turned out to be a very large gamble since the EPA refused to set threshold limits for LD 50's and LC 50's and agreed only to review the test results subjectively.

Due to the unusual length of the studies, I will read only the abbreviated results which finally passed the EPA Review Board in 1985 and resulted in registration after countless false starts. Margosan-0 was administered in undiluted form in these tests.

Avian Single-Dose Oral LD<sub>50</sub>: Margosan-0 was administered to Mallard ducks in order to determine a dose lethal to 50% of the duck population. Dose levels ranged from 1 to 16 ml/kg of body weight. All ducks remained active and healthy through the 14-day experimental period. The acute LC<sub>50</sub> of Margosan-0 is in excess of 16.0 ml/kg.

Avian Dietary LC<sub>50</sub> with Bobwhite Quail: The birds were given basal diet, with additions of Margosan-0 ranging from 1000 to 7000 ppm. Observations showed no negative effects and the quail were active and healthy throughout the 5-day period and the 3-day recovery phase. The acute oral LC<sub>50</sub> of Margosan-0 to Bobwhite quail is therefore in excess of 7000 ppm.

Avian Dietary LC<sub>50</sub> with Mallard Ducks: They were also given 1000 to 7000 ppm concentrate for 5 days and the ducks remained active and healthy during the test and recovery period. The acute LC<sub>50</sub> to Mallard ducks is therefore in excess of 7000 ppm.

Acute Toxicity of Margosan-0 to Rainbow Trout: The LC<sub>50</sub> was 8.8 ml of Margosan-0 per liter of water, and the 96 hour of no-observed-effect was 5 mg/l.

Acute Toxicity of Margosan-0 to Bluegill Sunfish: It showed 96-hour LC<sub>50</sub> of 37 mg/l and a no-observed-effect 96-hour level of 20 mg/l.

Acute Toxicity of Margosan-0 to Daphnia Magna, a Water Flea: The test was done on newly molted instars less than 20 hours old which were placed in a fresh aquatic habitat for up to 48 hours. The LC<sub>50</sub> was 13 mg/l and the no-observed-effect concentration at 48 hours was less than 10 mg/l. The toxicity value was well within the expected range, but it indicated that Margosan-0 may have some affect on primitive aquatic invertebrates under static conditions.

Acute Oral Toxicity: Rats were dosed once and then observed for 14 days for abnormal behavior or mortality. No negative effects were observed and the acute oral toxicity of the test material was in excess of 5 ml/kg, the limit of the required test.

Acute Dermal Toxicity on Albino Rabbits: No mortality resulted, and the acute dermal toxicity (LC<sub>50</sub>) of Margosan-0 was in excess of 2 ml/kg.

Skin Irritation: The results of Margosan-0 on Albino rabbits showed low to moderate primary irritation to shaved areas and high to moderate irritation in the abraded area.

Acute Inhalation Study: LC<sub>50</sub> of Margosan-0 on Albino rats was in excess of 43.9 mg/l/h, the limit of the test.

Modified Eye Irritation: Margosan-0 was administered to one washed eye and one unwashed eye of albino rabbits. Over 7 days both eyes showed minimal irritation.

Immune Response: Studies suggest that the test material does not cause adverse immune response.

Sensitization: This test was done on guinea pigs, which were shaved and patched with Margosan-0 for 6 hours. The procedure was repeated on alternate days for a total of nine applications. Margosan-0 did not produce sensitization.

Mutagenicity: The traditional Ames mutagenicity study was used in the U.S. on five strains of Salmonella typhimurium. Results showed that Margosan-0 concentrate is non-mutagenic.

Bee Adult Toxicity Test: This test was done voluntarily and was not ordered by the EPA. With the assistance of the University of California Apiary at Riverside, CA, Vikwood Ltd. ordered a toxicity test on honeybee worker adults. Margosan-0 was administered as a direct contact chemical using field dosages up to 4478 ppm a.i./ha. It was found to be benign to honeybees at well above the recommended dosage of 20 ppm (diluted, as a foliar spray) for a common pest, the gypsy moth, Lymantria dispar (L.).

## CONCLUSION

It has been a rare privilege to participate in so worthy a project for the environment and to have had the confidence and unstinting support of the USDA and truly be a partner of the NEEM TEAM.

Margosan-0 may well become one of the more important of the new wave of "soft" pesticides being developed with acute environmental concerns in mind, substituting for many of the "hard" pesticides now being withdrawn due to lasting, or as yet unknown damage to the environment.

## DEVELOPING A NEEM-BASED PEST MANAGEMENT PRODUCT,

J. F. Walter and J. F. Knauss

## ABSTRACT

The development of biocontrol products includes several key steps including: active ingredient identification, formulation development, process development and validation, efficacy and plant safety testing, toxicology testing, EPA registration, and market introduction. There are unique problems and opportunities presented by a neem extract that have been addressed in commercializing Margosan-OR insecticide.

## INTRODUCTION

The development of a neem-based pesticide at W. R. Grace is a relatively short story owing to the great amount of work already accomplished by researchers throughout the world. In 1988, Robert Larson approached W. R. Grace's Horticultural Products group with a proposition to commercialize a new neem-based pesticide identified by the trademark Margosan-O. While Larson had been able to patent and obtain EPA registration for his material he could not produce commercial quantities for sale. The activities and attributes of neem products and azadirachtin are well documented in the literature and are very impressive and yet no commercial insecticide neem products are available outside of India other than Margosan-O insecticide.

A cursory analysis of the situation revealed that there are several technical reasons why neem-based pesticides are not common; these include: the instability of azadirachtin, the variability of a natural raw material, inconsistency of final product and potential phytotoxicity. Beyond the technical problems, commercial uncertainties, such as organizing a constant source of neem seeds, pricing and compatibility with commercial practices were obvious. Yet, all these problems have been addressed in the further development of the commercial product, Margosan-O insecticide, reported here. This involved a six step process going from market analysis to introduction.

STEP 1--IDENTIFYING  
A TARGET MARKET

One of the difficulties in developing a neem-based pesticide was the observation that azadirachtin was reported to work on such a wide variety of pests (> 130 insects at last count) that it was necessary to pick a single target market and pest for testing. Due to Grace's Horticultural Products experience in the greenhouse market, we settled on that market and then went about identifying a target insect. We chose greenhouse and sweet potato whitefly due to the wide range of plants they attack and the difficulties growers have experienced in controlling these pests because of their rapid resistance development to synthetic pesticides. Our game plan was to focus our attention on these two pests while providing experimental material to academic researchers who wanted to explore other applications. A market analysis was conducted that identified potential market sizes and competitive products and pricing. From this we identified pricing constraints and market characteristics.

STEP 2--FORMULATION  
DEVELOPMENT

Many researchers have noted that azadirachtin is a relatively sensitive molecule that can easily degrade. Stokes et al. (1982) showed that it is sensitive to ultraviolet light, while Larson (1989) showed that azadirachtin stability is affected by solution pH and the concentration of azadirachtin in solution. Furthermore, Schumutterer (1990) notes that azadirachtin is more or less sensitive to temperature, ultraviolet light, pH, rainfall, and other environmental factors when applied to plant tissue. Coupled to these

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Walter is a manager, Biochemical Engineering, [W. R. Grace & Co.] Conn., 7379 Route 32, [Columbia, MD] 21044; Knauss is a director, Plant Protection Technology, Grace-Sierra, 570 Grant Way, Fogelsville, PA 18051.

limitations is the realization that for horticultural applications a formulation must be non-phytotoxic to most plant species and should be compatible with current application practices. Based on these factors and discussions with researchers in the azadirachtin area, we recognized that a reliable, reproducible, shelf-stable formulation was the key to developing an azadirachtin-based product.

Thus, the process to produce the material and the formulation are intimately tied together. We determined that an emulsifiable concentrate would be the most compatible with growers' operations and could be most efficiently produced. We formed a research team consisting of an organic chemist, analytical chemist, and a chemical engineer who, within nine months, identified the critical parameters that control azadirachtin stability and developed a series of formulations and processes that passed critical stability, handleability, and process constraints. These formulations were then tested in a "real world" environment.

#### STEP 3---INITIAL EVALUATIONS/DEVELOPING ESSENTIAL PARAMETERS

In the third stage of screening, real-life application and efficacy trials were conducted in a greenhouse. Essential to the further development of Margosan-0 insecticide was the ability to rapidly evaluate the prospective formulations in-house and/or with selected outside cooperators. For many initial and subsequent quick studies, the Grace-Sierra research greenhouse, located in Fogelsville, PA, was utilized.

Pest pressure in Fogelsville was maintained by an ongoing population of greenhouse whitefly, *Trialeurodes vaporariorum*. In addition, occasional natural infestations of several aphid and thrips species, along with the two-spotted mite, *Tetranychus urticae*, provided opportunities for efficacy evaluations.

These in-house evaluations helped formulate guidelines, such as: effective concentration levels, potential phytotoxic levels, activity range, and application timing relative to interval and time of day.

In addition, the greenhouse was shared with horticultural researchers whose plants were continually exposed to the ongoing whitefly population which was managed by preventative sprays of Margosan-0 insecticide on 7-10 day intervals. This activity covered a two-year and counting period during which the greenhouse whitefly susceptible plants exhibited excellent whitefly management when sprayed with Margosan-0 insecticide.

From the third stage tests, we identified four formulations that looked effective and met our stability requirements. Subsequently, we wanted our materials tested by non-biased academic researchers to determine if they were effective and compatible with current cultural practices.

#### STEP 4---DEVELOPING A COOPERATIVE NETWORK

In order to conduct the academic cooperative trials, we needed material to test, cooperative researchers, and test protocols. A mini pilot plant was set-up to produce several gallon batches of each formulation for testing.

A key component in any evaluation program is identifying outside researchers who can properly assist in the product evaluation and development process. This task proved easy because of the remarkably effective network that the USA entomologists working on greenhouse crops have established.

We relied upon eight prominent university and several industry entomologists to assist in formulation selection and later efficacy range testing. This work was accomplished largely through grants-in-aid to help with costs inherent in the evaluations.



Protocols were developed around previous knowledge that had been developed by the USDA-ARS in its initial work on Margosan-O insecticide in support of Larson's registration efforts. In addition, work in-house at Fogelsville, and initial studies by the researchers, allowed clarification of the essential components.

Once the effective product concentration range and proper methods for use were determined, the protocols were developed and the research network provided the data that clarified what Margosan-O insecticide controlled, what it did not affect, the effective concentration range, the proper manner for use and its acceptability in current application techniques.

#### STEP 5—COMMERCIAL FIELD TESTING

A mistake often made by companies anxious to get a product to market is not to properly field evaluate the product. This can be lethal as can be attested to by several recent introductions of biological/natural products that proved unacceptable when put to the rigors of commercial use. With Margosan-O insecticide, this phase of the development process was not short-changed.

In order to conduct effective commercial trials it became essential to produce several hundred gallons of material for testing. To produce this we constructed a pilot plant where scale-up issues were addressed and the reproducibility of the process validated.

Grower establishments, in several states, and coordinated through university researchers, evaluated Margosan-O insecticide during the 1989 poinsettia season for management of sweet potato and greenhouse whitefly. These evaluations provided, in addition to efficacy, phytotoxicity and acceptability performance in a variety of spray application systems.

Sprays were provided at suggested concentrations up to the point of plant sale. Results indicated remarkable plant safety and good-to-excellent control of the whitefly populations challenged. After we received back the data from these trials, we selected the formulation to bring to market.

This last evaluation step was the key in jumping the final hurdle to product launch.

#### STEP 6—MARKETING THE PRODUCT

Margosan-O insecticide, within the Grace-Sierra system, had now reached the point where Grace-Sierra Technical relinquished the product to Marketing.

The questions of product launch, product positioning, and all the other marketing concerns were now in the hands of those remarkable folks who provide the final touches to a product that has completed the six steps involved in evaluating technical performance.

#### REFERENCES

- Larson, R. O., 1989. The Commercialization of Neem, p. 155-168. In: Focus on Phytochemical Pesticides, M. Jacobson (ed.), Vol. I. The Neem Tree, CRC Press, Boca Raton, FL.
- Schmutterer, H., 1990. Properties and Potential of Natural Pesticides from the Neem Tree, *Azadirachta indica*. Ann. Rev. Entomol. 35:271-297.
- Stokes, J. B., et al., 1982. Effect of Sunlight on Azadirachtin Antifeeding Potency. J. Envir. Sci., Health A17(1), 57.



## DEVELOPMENT OF A NEEM-BASED INSECTICIDE IN CANADA

M.B. Isman, O. Koul, D.T. Lowery, J.T. Arnason, D. Gagnon, J.G. Stewart and G.S. Salloum

### ABSTRACT

Both laboratory and field studies have been conducted as part of a project aimed at development of a neem-based insecticide based on seed oil for the consumer market and specialty horticultural crops. To determine the importance of azadirachtin content for bioactivity, twelve samples of neem seed oil were analyzed for azadirachtin content via HPLC, and subjected to parallel bioassays which discriminate between behavioral (antifeedant) and physiological (molt-disrupting) effects. Azadirachtin content is highly correlated with bioactivity in all bioassays employing the variegated cutworm and the large milkweed bug. Field trials have been conducted for two seasons against Colorado potato beetle on potato, European corn borer on sweet corn, and diamondback moth and aphids on cabbage. Our experimental neem insecticide was not as efficacious as synthetic pyrethroids for control of the Colorado potato beetle but was so in both trials with the corn borer. In both the potato and cabbage trials, the neem insecticide was as effective or better than natural pyrethrum, the current choice amongst organic growers.

### INTRODUCTION

The biological activities of extracts of the neem tree, *Azadirachta indica* A. Juss. (Meliaceae), and its principle active constituent, azadirachtin, were 'discovered' almost two decades ago in European laboratories (Butterworth and Morgan, 1968; Leuschner, 1972; Ruscoe, 1972), and the potential for commercial application of azadirachtin or neem-based insecticides and antifeedants has been recognized for at least a decade (Schmutterer et al., 1981). In the interim, numerous laboratories worldwide have investigated the chemistry, biological activity, and efficacy of neem derivatives, the results of which can be found in several volumes and reviews (Schmutterer and Ascher, 1984, 1987; Jacobson, 1989; Schmutterer, 1990). It is perhaps somewhat surprising then, that only a single neem-based insect control product (Margosan-O<sup>TM</sup>) has been registered for use in North America, and its commercial use is just beginning this year.

However, as environmental issues continue their mercurial rise amongst the general public, and the regulatory climate in both the United States and Canada appears set for change to accommodate environmentally-sound pest control materials developed by smaller companies, the acceptability and market potential for neem-based insecticides should rise accordingly, with room for several such products in the marketplace. Safer Ltd., whose mandate for over a dozen years has been the development and sale of environmentally-sound yet effective pest control products, has been in the process of developing neem-based insecticides in Canada in collaboration with scientists at the University of British Columbia, the University of Ottawa, and Agriculture Canada. In the present paper, we review our laboratory findings and field studies completed to date which indicate our approach to the development of a viable neem-based product.

Following initial studies at the university laboratories on the effects of azadirachtin and its mode of action against various insects (Arnason et al., 1985; Champagne et al., 1989), we began testing a concentrated neem-seed kernel extract. Several considerations, though, led us to change the course of our project and instead adopt neem seed expeller oil (the viscous, brown oil produced by simple crushing of the seed kernels) as the favored starting material for development of a commercial insecticide/antifeedant. These included the potential contribution of other active constituents in the oil, the potential stabilizing effect of the oil on azadirachtin (through natural antioxidants and UV-absorbing substances), the fact that the oil itself may have biological activity against some soft-bodied insects (comparable to other 'horticultural' oils), and the relative abundance and availability of neem oil in India. In addition, we obtained a sample of a neem insecticide currently marketed in India which our analysis proved to be a formulation of crude neem oil.

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Isman, Koul and Lowery are associate professor, research associate and graduate student, respectively, Department of Plant Science, University of British Columbia, Vancouver V6T 2A2, Canada; Arnason and Gagnon are professor and graduate student, respectively, Biology Department, University of Ottawa, Ottawa K1N 6N5, Canada; Stewart is a research scientist, Agriculture Canada Research Station, Charlottetown, P.E.I. C1A 7M8, Canada; and Salloum is an entomologist, Safer Ltd., 6761 Kirkpatrick Crescent, R.R. 3, Victoria, B.C. V8X 3X1, Canada.

Our earliest studies focussed on the effects of pure azadirachtin against two polyphagous lepidopterans, the variegated cutworm (*Peridroma saucia* Hubner, Noctuidae) and the European corn borer (*Ostrinia nubilalis* Hubner, Pyralidae). Addition of azadirachtin to artificial media strongly suppressed growth of both species. For the cutworm, we established an  $EC_{50}$  (dietary concentration reducing larval growth to 50% of controls) for neonate larvae of 0.26 ppm, which makes it more than ten fold more active than the next most active natural product bioassayed against this species, and 100-1000 times more active than most natural growth inhibitors (e.g. sesquiterpene lactones, Isman et al., 1989). In the cornborer, the  $EC_{50}$  was not precisely established, but was within the range from 0.1 - 1.0 ppm in the diet. This type of bioassay does not discriminate between behavioral and physiological causes of impaired growth, and therefore antifeedant effects of azadirachtin were assessed directly using a leaf-disc choice test. Feeding deterrence in these (and undoubtedly other) species varies with age, but we found that the variegated cutworm is quite sensitive to azadirachtin, with an  $EC_{50}$  (concentration reducing leaf area consumption to 50% of controls) for fourth instar larvae of 2.4 ppm ( $=8.0 \text{ ng/cm}^2$ ). Earlier instar larvae are significantly more sensitive.

We know, however, that the antifeedant effects of azadirachtin do not alone account for larval growth inhibition in these lepidopterans. For both species, nutritional analysis experiments with later instars indicated that growth is inhibited largely through reductions in dietary utilization (conversion of ingested food to biomass) (Arnason et al., 1985; Koul and Isman, unpublished data). Furthermore, dietary concentrations of this compound which are not inhibitory to larval growth nonetheless can result in significant mortality in the final instar prior to pupation (*P. saucia*) and severely reduce fecundity of surviving adult moths (*O. nubilalis*).

Given that the desert locust (*Schistocerca gregaria* Forsk., Acrididae) is perhaps the most sensitive insect to the antifeedant action of azadirachtin, it was surprising to find that a major North American grasshopper pest, the migratory grasshopper (*Melanoplus sanguinipes* Fab., Acrididae) was undeterred, feeding readily on cabbage leaf discs treated with up to 500 ppm azadirachtin. However, azadirachtin consumed by fifth instar nymphs of *M. sanguinipes* has a clear molt-disrupting effect which is dose dependent (Champagne et al., 1989). We were able to establish  $LD_{50}$  values (lethal dose based on disruption of the terminal molt) of  $11.3 \mu\text{g/g}$  insect fwt (approx.  $1.4 \mu\text{g/nymph}$ ) orally, and  $4.5 \mu\text{g/g}$  fwt ( $=0.56 \mu\text{g/nymph}$ ) topically, indicating excellent contact action *via* the integument. However, the large milkweed bug (*Oncopeltus fasciatus* Dallas, Lygaeidae), an insect commonly used in studies of insect growth regulators (IGRs) because of its sensitivity to endocrine disruption, is over 30 times more susceptible than the grasshopper to molt-disruption from topically-applied azadirachtin. For 5th instar *O. fasciatus*, we established an  $ED_{50}$  (effective dose producing 50% molt disruption relative to controls) of  $0.14 \mu\text{g/g}$  fwt ( $=3.5 \text{ ng/nymph}$ ). Because of its extreme sensitivity to azadirachtin, we have used this insect to evaluate neem samples and extracts from other meliaceous species for IGR activity.

#### Chemical and Biological Evaluation of Neem Oils

Although azadirachtin can be both isolated in purity from neem (Schroeder and Nakanishi, 1987) and totally synthesized in the laboratory (Ley, 1989), production of pure azadirachtin on a commercial scale appears prohibitively expensive. As a result, all of the neem-based insecticides commercialized in India and Margosan-O<sup>TM</sup> in the U.S.A. are based on neem seed oils or extracts. Neem oils and to a lesser extent, extracts, are readily available from import from India (and some other countries). However, neem, like any other natural substance, is expected to vary widely with respect to its active principles. Are all neem oils equally efficacious?

We sought to establish the possible significance of azadirachtin as a quality control criterion for evaluating sources of neem oil as an insecticide precursor by analyzing different neem oil samples for azadirachtin content, coupled with parallel bioassays of the oils (Isman et al., 1990a). We used reverse-phase gradient liquid chromatography (HPLC) to quantify azadirachtin content in a dozen samples of neem oil obtained from various sources in India. We found an extremely wide spectrum of azadirachtin concentrations: two of the oils lacked detectable amounts of azadirachtin (limit of detection approx. 50 ppm), while the remaining ten ranged from roughly 200-4000 ppm (0.02-0.4%) (Figure 1). More recently we analyzed one neem oil sample containing approximately 6800 ppm azadirachtin.

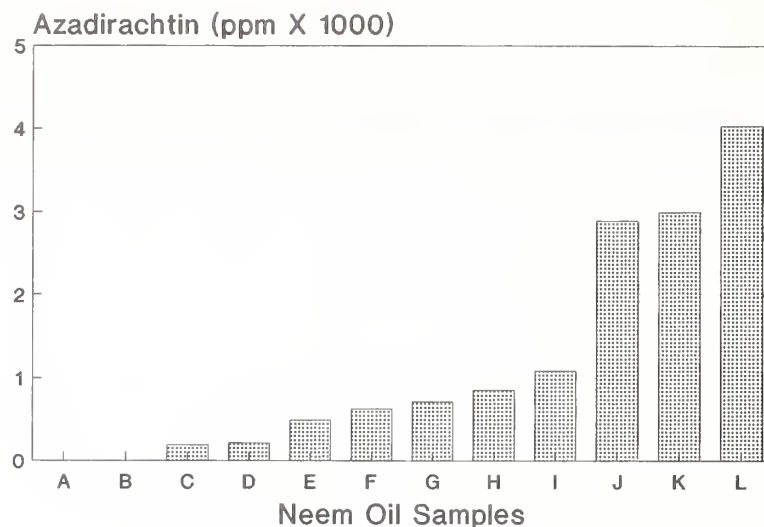


Figure 1  
Azadirachtin content of twelve neem oil samples as determined by HPLC.

The oils were subjected to three different bioassays. The first involved adding neem oils at different concentrations to an artificial medium on which neonate variegated cutworms were placed. Larvae then fed on the diets for seven days, after which they were weighed. As previously mentioned, inhibition of larval growth in this bioassay can arise through either behavioral or physiological mode of action (or both). To distinguish between these possibilities, two additional bioassays with more specific endpoints were utilized. To assay for antifeedant effects, fifth instar cutworm larvae were offered cabbage leaf discs treated with neem oils and leaf discs treated with the carrier solvent alone. Larvae were allowed to feed in this choice test for six hours, after which leaf area consumption was measured with a digitizing area meter. The final bioassay assessed IGR (molt-disrupting) effects exclusively. Neem oils were applied topically to last instar milkweed bug nymphs (with appropriate carrier controls) and then bugs were held with food and water for at least 10 days. Bugs were observed and scored on the basis of successful or unsuccessful molting to the adult stage. Each of the twelve oils was tested in each of the bioassays at four or five concentrations, allowing calculations of  $EC_{50}$  values (concentration resulting in 50% response relative to controls).

For our bioassay species, biological activity (both behavioral and physiological) is highly related to azadirachtin content of the neem oils (Isman et al., 1990a)(Table 1). Variation in azadirachtin content in neem oil accounts for 72-90% of the variation in biological activity of the oils. There are undoubtedly other insect species for which this correlation would not be significant, but the striking similarity between our results and those of Ermel et al. (1987), comparing IGR activity of neem seed extracts against the Mexican bean beetle, (*Epilachna varivestis* Mulsant, Coccinellidae) is noteworthy. One case where we did not find a correlation between bioactivity and azadirachtin content was in feeding choice tests with aphids. Neem oil at 1% in solution applied to leaf discs deters feeding in five species of aphids, the strawberry aphid *Chaetosiphon fragaefolii* Cockerell (Aphididae) being most sensitive, and the lettuce aphid *Nasonovia ribisnigri* Mosley, the least. Comparison of antifeedant activity of different neem oils against the strawberry aphid indicated that bioactivity of the oils was dose-dependent, but independent of azadirachtin content, and may instead result from the presence of volatile sulfides (Balandrin et al., 1988) in the oils.



Table 1  
Bioactivity of neem oils:  $EC_{50}$  versus azadirachtin content

Bioassay	r =	P
<i>Peridroma</i> neonate chronic growth	-0.92	< 0.0001
<i>Peridroma</i> 5th instar choice test	-0.85	< 0.001
<i>Oncopeltus</i> molt disruption	-0.95	< 0.0001

N = 10 oils;  $EC_{50}$ s (log-transformed) based on 4-5 concentrations of each oil.

## FIELD STUDIES

For the past two years, we have been evaluating prototype neem-based insecticides, developed by Safer Ltd., on vegetable pests under field conditions in eastern, central and western Canada. Our program of field trials will continue in 1990. The present paper reviews the results of our 1989 trials.

### Potato

In 1989, two field trials were conducted at Prince Edward Island to evaluate the efficacy of Safer's experimental neem insecticide (ENI) relative to other natural insecticides (pyrethrum, *Bacillus thuringiensis* var. tenebrionis [M-One™]) and currently recommended synthetic pyrethroids (deltamethrin, esfenvalerate) for control of the Colorado potato beetle (*Leptinotarsa decemlineata* Say, Chrysomelidae). In the first experiment (Stewart et al., 1989), neither formulated crude neem oil nor formulated pyrethrum provided adequate control; yields of marketable tubers were no better than for untreated plots (Figure 2). Plots treated with Safer's ENI produced significantly greater yield, and though not as great as that produced in plots treated with the synthetic pyrethroid, yields did not differ significantly between these two treatments. What is particularly interesting is that the plots treated by the ENI harbored significantly larger densities of beetles (especially larvae) compared to plots treated with the pyrethroid, yet the ENI plots produced acceptable yield in spite of the presence of the insects. We have observed a similar situation in our field trials on sweet corn (next section).

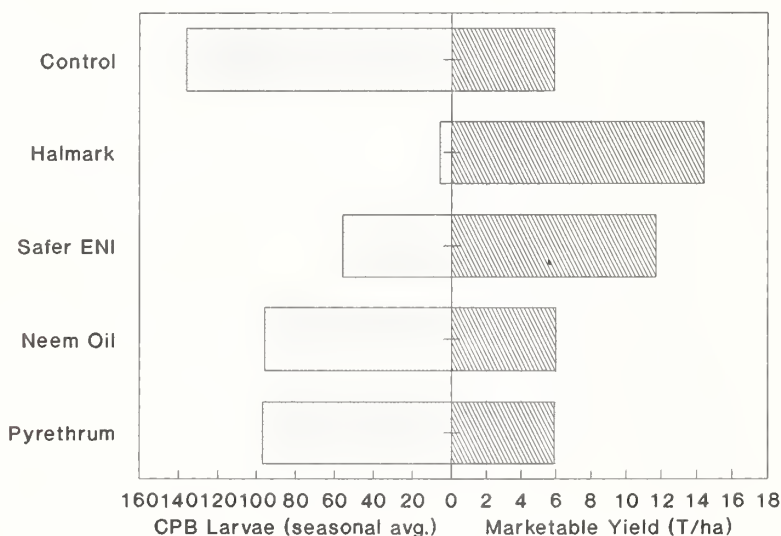


Figure 2  
Numbers of Colorado potato beetle larvae per 10 sweeps (seasonal average) and marketable yield of tubers in potato plots treated with different insecticides at Charlottetown, P.E.I., Canada. Plots treated with Halmark were sprayed two times; for other treatments five applications were made.

In the second experiment (Stewart and Thompson, 1989) ENI-treated plots produced yields equal to those from plots treated with the microbial insecticide (M-One™), although neither produced yields as great as those resulting from treatment with the synthetic pyrethroid deltamethrin. Collectively though, our results indicate that the neem-based insecticide is at least as effective as other 'natural' pest control materials for control of this important pest.

### Corn

We have been conducting field trials at Ottawa, Ontario comparing the efficacies of a neem seed extract and Safer's ENI with a recommended synthetic pyrethroid, permethrin, for control of the European corn borer on sweet corn. In these trials, corn ears were artificially infested with early instar larvae to simulate years of highly-damaging natural infestations, because the appearance of this insect is sporadic and unpredictable in this region. Both of the neem treatments significantly reduced damage (Figure 3), being as efficacious as the synthetic pyrethroid, whereas the ENI gave superior control. It is noteworthy that a single application of neem prior to infestation provides good protection, and additional sprays after infestation do not significantly enhance the protection. In the previous year's trials, we found that a single application pre-infestation was as effective as four applications following infestation (Isman et al., 1990b). As was noted in the potato trials, ears treated with neem contained substantially more larvae than those treated with permethrin, although the neem-treated ears did not sustain more damage (Figure 3), which supports the hypothesis that neem has an IGR effect on larvae which persists after they have entered the ear, even if no longer exposed to the toxicant.

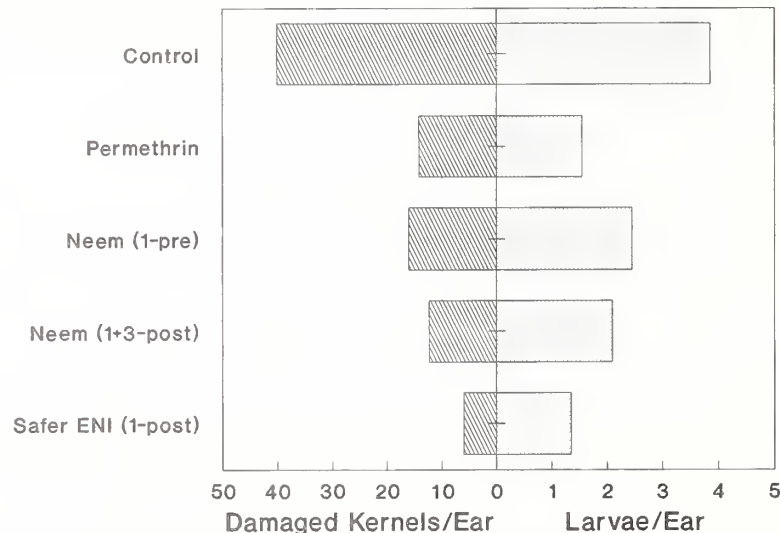


Figure 3  
Numbers of European corn borers and number of damaged kernels per ear in sweet corn plots treated with different insecticides at Ottawa, Ontario, Canada. Corn ears were artificially infested with young larvae. '1-pre' = 1 application prior to infestation; '1 + 3-post' = 1 application prior to infestation and 3 applications thereafter.

### Cabbage

In 1989 we conducted a field trial at Vancouver comparing the efficacy of Safer's ENI, formulated crude neem oil, and formulated pyrethrum for control of aphids and lepidopterous pests. Treatments were applied three times at weekly intervals. All of the treatments significantly reduced natural infestations of the diamondback moth (*Plutella xylostella* L., Plutellidae); two applications of either pyrethrum or the ENI were sufficient for almost complete control of this insect (Figure 4). Destructive sampling of the plots ten days following the third application revealed that the neem oil- and ENI-treated plots had significantly fewer larvae than the pyrethrum-treated plots (Isman et al., 1990b). Damage to cabbage heads based on semi-quantitative visual assessment was only significant on pyrethrum-treated and untreated plots. No other lepidopterous pests reached damaging levels in the field trial. For the current field season (1990) we intend to evaluate neem both in naturally-infested plots and in plots artificially-infested with diamondback moths, imported cabbageworms (*Pieris rapae* L., Pieridae), cabbage loopers (*Trichoplusia ni* Hubner, Noctuidae), and bertha armyworms (*Mamestra configurata* Walker, Noctuidae) if necessary.



Both green peach aphids (*Myzus persicae* Sulzer) and cabbage aphids (*Brevicoryne brassicae* L.) were effectively controlled by the ENI and the crude neem oil, but pyrethrum was ineffective against both species (Figure 5). Additional smaller scale field trials and greenhouse trials were conducted using other host/aphid combinations. On head lettuce, both Safer's ENI and pyrethrum significantly reduced numbers of lettuce aphids by approximately 80% relative to controls. In the greenhouse, single applications of neem oil prior to artificial infestation resulted in only 50% reductions in populations of lettuce and strawberry aphids on their respective hosts, whereas a single application on rutabaga reduced subsequent populations of green peach aphids by approximately 85% (Lowery and Isman, unpublished data). A single application of ENI to field collards prior to infestation with cabbage aphids reduced populations by 65% relative to controls (F. Campos, unpublished data).

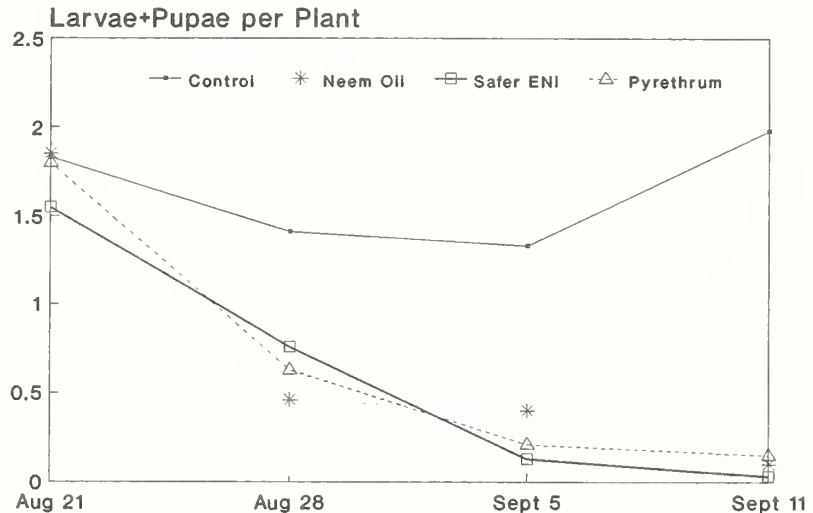


Figure 4  
Effect of different insecticides on natural infestations of diamondback moth (larvae and pupae) on cabbage at Vancouver, B.C., Canada. Treatments were applied August 23, 31 and September 7.

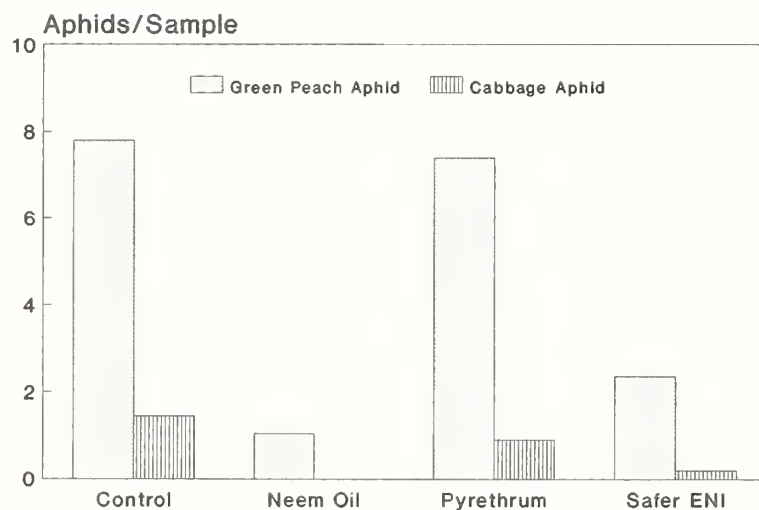


Figure 5  
Effect of different insecticides on natural infestations of green peach aphid and cabbage aphid on cabbage at Vancouver, B.C., Canada. Treatments were applied three times, and the four outermost leaves on each plant sampled for aphids ten days after the last application.

Although control of aphids by neem varies considerably between species, for some species (especially the difficult-to-control green peach aphid) neem-based insecticides may be quite effective, in spite of the relatively poor deterrent/antifeedant effect seen in laboratory bioassays. Together, these laboratory and field results point to possible IGR effects of neem (with azadirachtin as the presumed active constituent) against aphids as the source for efficacy in the field (population reductions). To that end we have initiated laboratory investigations of the physiological effects of neem against a number of aphid species. To date we have observed that both neem oil and pure azadirachtin when applied to leaf discs result in high rates of mortality for second and fourth instar lettuce aphids (Lowery and Isman, unpublished data). While neem and azadirachtin do not result in mortality of adult aphids, fecundity is dramatically reduced, as well as the viability of offspring.

## COMMERCIAL PROSPECTUS

The results of our field trials, which make a small contribution to the already overwhelming body of literature on the field efficacy of neem-based crop protectants, provide further evidence that neem-based insecticides have tremendous potential for control of insect pests in North America. We expect that 'organic' food producers, who at present depend on pyrethrum, rotenone and *Bacillus thuringiensis* for insect control, will alone constitute an important market niche for neem insecticides. We have already demonstrated that Safer's ENI performs as well or better than pyrethrum in several crop/pest contexts. We believe that neem-based insecticides will be widely accepted by the general public because of their natural origin, lack of mammalian toxicity, and minimal environmental impact. Proposed changes in regulatory requirements for 'environmentally-sound' pest control materials by both the Environmental Protection Agency (U.S.A.) and the Pesticides Directorate of Agriculture Canada should expedite the transfer of neem-based insecticides from the laboratory into the marketplace.

We anticipate that neem will have a significant impact in domestic use (home and garden) as well as in 'organic' agriculture, because in these situations the demand for cosmetically perfect and insect-free produce is relaxed. The ability of neem to displace synthetic insecticides currently used in large scale commercial food production systems may depend largely on the development of neem products with maximum azadirachtin content, and knowledge of the most appropriate means and timing of applications to ensure optimal efficacy. As neem for the most part lacks acute toxicity to most insects, there are undoubtedly going to exist some pest control contexts where this material will not provide adequate control, for example where industry imposes 'zero'-tolerances for insects and/or damage, or where immediate population suppression is required to prevent economic loss. However, in both our corn and potato trials, we have shown that acceptable yields can be obtained without acute toxicity to the target pests.

Other key market niches for neem-based products may be found amongst specialty horticultural crops (certain vegetables and small fruits) for which there are few, if any, registered chemicals which are effective. Many of the multinational companies are no longer supporting registrations on specialty crops, which for them constitute too small a sales market to service. This situation is expected to be further aggravated as current registrations lapse in the absence of suitable replacement products.

As public demand for more environmentally-sound pest control materials and methods grows, there should be a commensurate market for neem-based insecticides in the 1990s. To that end, neem may be on the leading edge of a wave of new pest control materials of plant origin.

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## REFERENCES

- Arnason, J.T., B.J.R. Philogene, and nine others. 1985. Antifeedant and insecticidal properties of azadirachtin to the European corn borer, *Ostrinia nubilalis*. Entomol. exp. appl. 38: 29-34.
- Balandrin, M.F., S.M. Lee, and J.A. Klocke. 1988. Biologically active volatile organosulfur compounds from the seeds of the neem tree, *Azadirachta indica* (Meliaceae). J. Agric. Food Chem. 36: 1048-1054.

- Butterworth, J.H. and E.D. Morgan. 1968. Isolation of a substance that suppresses feeding in locusts. *J. Chem. Soc. Chem. Commun.*, pp. 23-24.
- Champagne, D.E., M.B. Isman, and G.H.N. Towers. 1989. Insecticidal activity of phytochemicals and extracts of the Meliaceae, pp. 95-109. *In* J.T. Arnason, B.J.R. Philogene and P. Morand (eds), *Insecticides of Plant Origin*. American Chemical Society Symposium Series No. 387.
- Ermel, K., E. Pahlich and H. Schmutterer. 1987. Azadirachtin content of neem kernels from different geographic locations, and its dependence on temperature, relative humidity and light, pp. 171-184. *In* H. Schmutterer and K.R.S. Ascher (eds), *Natural Pesticides From the Neem Tree and Other Tropical Plants*. Schriftenreihe der GTZ, No. 206.
- Isman, M.B., N.L. Brard, J. Nawrot and J. Harmatha. 1989. Antifeedant and growth inhibitory effects of bakkenolide-A and other sesquiterpene lactones on the variegated cutworm, *Peridroma saucia* Hubner (Lep., Noctuidae). *J. Appl. Ent.* 107: 524-529.
- Isman, M.B., O. Koul, A. Luczynski and J. Kaminski. 1990a. Insecticidal and antifeedant bioactivities of neem oils and their relationship to azadirachtin content. *J. Agric. Food Chem.*, in press.
- Isman, M.B., O. Koul, and nine others. 1990b. Developing a neem-based insecticide for Canada. *Mem. Entomol. Soc. Canada*, in press.
- Jacobson, M. (ed). 1989. *Phytochemical Pesticides, Volume 1. The Neem Tree*. CRC Press, Boca Raton, 178 pp.
- Leuschner, K. 1972. Effect of an unknown substance on a shield bug. *Naturwissenschaften* 59: 217.
- Ley, S.V. 1989. Synthesis of insect antifeedants related to azadirachtin. Paper presented at Pacificchem '89, Honolulu, Hawaii.
- Ruscoe, C.N.E. 1972. Growth disruption effects of an insect antifeedant. *Nature* 236: 159-160.
- Schmutterer, H. 1990. Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. *Annu. Rev. Entomol.* 35: 271-297.
- Schmutterer, H. and K.R.S. Ascher (eds). 1984. *Natural Pesticides From the Neem Tree and Other Tropical Plants*. Proc. 2nd Intl. Neem Conf., Rauischholzhausen, 1983. Schriftenreihe der GTZ, No. 161, 587 pp.
- Schmutterer, H. and K.R.S. Ascher (eds). 1987. *Natural Pesticides From the Neem Tree and Other Tropical Plants*. Proc. 3rd Intl. Neem Conf., Nairobi, 1986. Schriftenreihe der GTZ, No. 206, 703 pp.
- Schmutterer, H., K.R.S. Ascher and H. Renibold (eds). 1981. *Natural Pesticides From the Neem Tree (Azadirachta indica A. Juss)*. Proc. 1st Intl. Neem Conf., Rottach-Egern, Fed. Rep. of Germany, 297 pp.
- Schroeder, D.R. and K. Nakanishi. 1987. A simplified isolation procedure for azadirachtin. *J. Nat. Prod.* 50: 241-244.
- Stewart, J.G. and L.S. Thompson. 1989. Residual activity of new insecticides for control of Colorado potato beetle. Research Summary of the Charlottetown Research Station, Agriculture Canada, p. 67.
- Stewart, J.G., L.S. Thompson, J.E. Lund, J. Arnason, M. Isman and G.S. Salloum. 1989. New insecticide compounds to control Colorado potato beetles on potatoes, 1989. Pesticide Research Report, Agriculture Canada, p. 92.

## Field Evaluations with Neem

### NEEM'S INSECTICIDAL AND ACARICIDAL ACTIVITY AGAINST FIELD CROP AND ORCHARD PESTS

K.R.S. Ascher

#### ABSTRACT

A brief review is given of some of the studies on the insecticidal effects of various neem preparations conducted at the ARO, The Volcani Center, Bet Dagan, Israel. The efficacy of neem seed kernel suspension (NSKS) or neem seed kernel extracts (NSKEs) prepared with different extracting solvents, and of azadirachtin (AZA) against various insect species, was investigated. The species included in the assays were: Boarmia selenaria, Epilachna varivestis, Spodoptera littoralis, Earias insulana, Ostrinia nubilalis, Sesamia nonagrioides, Liriomyza trifolii, and Eyprepocnemis plorans. Some other arthropods were also included in the project: the phytophagous mite Tetranychus cinnabarinus; the predacious mite Phytoseiulus persimilis; and the hunting spider Chiracanthium mildei.

#### INTRODUCTION

In the last few years, several books on neem (Schmutterer and Ascher, 1987; Jacobson, 1989) and an increasing number of review articles in books and journals on this subject (e.g. Jacobson, 1987; Saxena, 1989; Schmutterer, 1987, 1988, 1990) authored by prominent figures on the neem scene - Martin Jacobson, Ramesh C. Saxena, and in particular Heinrich Schmutterer - have appeared.

Israel has a relatively long history of research on the insect antifeedant properties of Indian lilac or neem (Azadirachta indica; Melia indica; syn. Antelaea azadirachta) and Persian lilac (Melia azedarach). In a lecture at the Third International Neem Conference in Nairobi (Ascher, 1987) I reviewed the very early pioneering work of Rachel Shpan-Gabrielith on M. azedarach concoctions against the desert locust, Schistocerca gregaria. In the latter half of the sixties, David Lavie et al. (1967) at the Weizmann Institute of Science in Rehovot, Israel, reported the isolation of the antifeedant meliantriol from both Melia species. Some results of work on neem at The Volcani Center in Bet Dagan, Israel, mainly with insect pests of field crops and also with phytophagous and predacious mites, are presented herewith: In an early study (Meisner et al., 1976) it was found that the giant spanner Boarmia (Ascotis) selenaria, a serious pest of avocado plantations in Israel and on many host plants, including field crops, elsewhere, is affected by 0.3% aqueous neem seed kernel suspension (NSKS), when its larvae are allowed to feed on sprayed potted avocado plants. Neem extractive or neem oil was ineffective. Furthermore, with larvae of B. selenaria kept for 8 days on NSKS-treated potted avocado plants, a similar residual effect was obtained when the residues were aged in the laboratory, or outside in constant shade under an arcade. If kept in the sun, however, the residues deteriorated rapidly.

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Ascher is an insect toxicologist, Department of Toxicology, Institute of Plant Protection, Agricultural Research Organization, (ARO), The Volcani Center, Bet Dagan 50-250, Israel. Contribution from the ARO. No. 3028-E.



In work done by the author in West Germany (Ascher, 1981), in Prof. Schmutterer's laboratory at Giessen, it was shown that by feeding adult females of the Mexican bean beetle, Epilachna varivestis, for 3 days bean seedlings sprayed with 0.25% dried methanolic neem seed kernel extract (NSKE), both egg fertility and fecundity were strongly reduced. Treatment of males only had no such effect and treatment of both sexes yielded the same results as treatment of females only. Furthermore (Ascher and Gsell, 1981), feeding of NSKE-sprayed bean seedlings to early L-4 larvae of E. varivestis produced striking IGR (insect growth regulatory) effects expressed by high larval mortality and development of the surviving pupae to abnormal adults, with varying degree of abnormality (Table 1).

Table 1  
Larval and pupal mortality and occurrence of abnormal adults due to a 3-day exposure of Epilachna varivestis fourth-instar larvae to neem seed kernel extract (NSKE) residues on beans. All percentages are calculated on the basis of the initial number of larvae in the experiment.

NSKE, concn (mg/l)	Dead larvae (%)	Pupae		Adults	
		Dead (%)	Live (%)	Abnormal (%)	Normal (%)
0	12.3	2.0	85.7	2.7	83.0
5	3.0	1.0	96.0	15.0	81.0
10	10.0	4.0	86.0	26.0	60.0
15	18.0	8.0	74.0	62.0	12.0
20	62.5	10.0	27.5	26.0	1.5
25	68.0	10.5	21.5	20.5	1.0
30	98.0	1.0	1.0	0.3	0.7

Several Israeli local species of cotton insects are strongly affected by neem products. Spodoptera littoralis, the Egyptian cotton leafworm, figures prominently among them. Fodder beet sprayed in the field with 1% emulsions of methanolic NSKE had a pronounced antifeedant and an intense IGR effect on S. littoralis larvae which were fed on it for 48 hours and then transferred to untreated food, especially with 1- and 3-day-old residues (Meisner et al., 1983). This led us to investigate different NSKEs extracted with a series of polar and apolar solvents (Ascher et al., 1984). When the Spodoptera larvae were fed for 2 days on alfalfa treated with the various extracts, only the extracts obtained with strongly polar solvents, viz., water, methanol, ethanol and acetone, showed activity in preventing pupation (Table 2).



Table 2  
Feeding 30-50 mg Spodoptera littoralis larvae for 2 days with  
alfalfa treated with various neem extracts: Effect on pupation.

Extracting solvents	Dielectric constant (T = 25°C)	% Neem extract in the dipping liquid		
		0.05	0.01	0.005
		% Pupation (corrected)		
Water	78.5	0	56	78
Methanol	32.6	0	30	78
Ethanol	24.3	1	51	98
Acetone	20.7	4.5	51	93
Isopropyl alcohol	18.3	80	98	
Butanol	17.1	89	80.5	91
Chloroform	4.8	97	100	
Carbon tetrachloride	2.2	93	93.5	
<u>n</u> -Pentane	1.8	86		

The extracts obtained with less polar or apolar solvents, i.e., with progressively lower dielectric constants, were practically inactive. The same rule for activity held true for adult emergence (Table 3), not only for the 0.05% but also for the 0.01% treatment.

Table 3  
Feeding 30-50 mg Spodoptera littoralis larvae for 2 days  
with alfalfa treated with various neem extracts:  
Effect on adult emergence.

Extracting solvents	Dielectric constant (T = 25°C)	% Neem extract in the dipping liquid		
		0.05	0.01	0.005
		% Adult emergence (corrected)		
Water	78.5	0	9+13 abn.*	70
Methanol	32.6	0	8+2 abn.	66
Ethanol	24.3	1	28	92
Acetone	20.7	2.5+2 abn.	26.5	88
Isopropyl alcohol	18.3	74	85	
Butanol	17.1	86	79	86
Chloroform	4.8	95	100	
Carbon tetrachloride	2.2	85	85	
<u>n</u> -Pentane	1.8	86		

\* abn. = abnormal

As a consequence, the methanolic extract was investigated intensively with different sizes of S. littoralis larvae, and was shown to prevent strongly pupation and emergence of adults. At low concentrations, in which a sufficient number of adults survived after the 2-day larval feeding with neem, fecundity was reduced heavily. It was also demonstrated (Meisner and Ascher, 1984) that another neem product, neem extractive, fed to larvae for 2 days along with treated alfalfa, had a rather strong IGR effect, preventing larvae from molting normally. Aqueous NSKS was much less active in this sense and neem oil was inactive. Finally (Meisner et al., 1981), the antifeedant effect of these products on S. littoralis larvae was estimated in field trials in addition to laboratory experiments. All three products at 0.6% on alfalfa showed good residual anti-feeding efficacy after 24 hours. On cotton, practically no protection was afforded by 0.6% of any of the products tested. On sugar beet, NSKS had the strongest residual antifeedant activity, for a few days.

Larvae, 7-8 and 12 days old, of another cotton insect, the spiny bollworm, Earias insulana, when fed on a semi-synthetic diet into which NSKS had been incorporated (the diet contained 0.075-1% of neem dry matter), died within 9 days (Meisner et al., 1978). The systemic antifeedant activity of neem against 7-day-old larvae of E. insulana was investigated with cotyledons of cotton stood for 24 hours in different concentrations of NSKS; at both 0.5% and 0.1% neem, larval weight gain after 3 days was about half that of control.

A further important field crop pest insect investigated was the European corn borer, Ostrinia nubilalis. Two- to 3-mg larvae of this insect were allowed to feed for 3 days on sweet corn seedlings sprayed with an aqueous solution of dried aqueous NSKE (Meisner et al., 1985), and then transferred to a semi-synthetic diet (Table 4).

Table 4  
Effects of different concentrations of an aqueous NSKE (fresh, 0-day-old residues) on the development of Ostrinia nubilalis (30 larvae per experiment; larval survival and average weight were determined 13 days after the start of the experiment).

Concentration of neem extracts (%)	Treated plants		Untreated plants	
	Survival of larvae (%)	Pupation (%)	Survival of larvae (%)	Pupation (%)
1.0	0*	0	73.3	73
0.5	0*	0	73.3	56
0.25	0*	0	80.0	50
0.1	10.0*	7	83.3	76
0.05	36.6	16	60.0	53

\* Significantly different from untreated plants at P=0.05 (Duncan's Multiple Range Test).

No pupation occurred on the 0.25% treatment, only 7% on the 0.1%, and 16% on the 0.05% treatment. The residual effect of this spray at 1% was investigated 2, 6 and 8 days after treatment (Table 5).

Table 5

Residual effect of 1% aqueous NSKE sprayed on corn seedlings, on the development of Ostrinia nubilalis (30 larvae per experiment; larval survival and average weight were determined 13 days after the start of the experiment).

Infestation of plants, days after treatment	Treated plants		Untreated plants	
	Survival of larvae (%)	Pupation (%)	Survival of larvae (%)	Pupation (%)
2	40.0*	0	66.6	60
6	30.0*	0	90.0	80
8	53.3	0	70.0	57

\*Significantly different from untreated plants at  $P=0.05$  (Duncan's Multiple Range Test).

Larval survival on 2- and 6-day old residues was significantly lower than on control plants, and the average weight of the larvae in the treatments 13 days after the beginning of the experiments was 1/7, 1/8 and 1/8 of that of the control, on 2-, 6- and 8-day-old residues, respectively; no pupation occurred in any of these treatments, even on 8-day-old residues. Systemic properties (Table 6) were demonstrated by incorporating neem extract into the soil in flower pots in which corn had been planted; the plants were infested for 3 days with larvae, 2, 4 and 6 days after the soil treatment.

Table 6

Plant systemic effect of 1% aqueous NSKE on the development of Ostrinia nubilalis (30 larvae per experiment; larval survival and average weight were determined 13 days after the start of the experiment).

Infestation of plants, days after soil treatment	Treated plants			Untreated plants		
	Survival of larvae (%)	Average weight/ larva (mg±S.E.)	Pupation (%)	Survival of larvae (%)	Average weight/ larva (mg±S.E.)	Pupation (%)
2	80	33.8±5.0	50	80	50.1±4.5	70
4	70	22.0±3.4*	10	77	47.4±6.1	63
6	66	35.3±3.9*	23	80	46.0±1.5	56

\*Significantly different from untreated plants at  $P=0.05$  (Duncan's Multiple Range Test).

Larval weight gain after 13 days was in all three cases significantly lower than in the control. The pupation rate in the 4- and 6-day treatments was very low in comparison with the controls. Similar experiments with corn seedlings were conducted on *O. nubilalis* with azadirachtin (Meisner et al., 1986a). With the 3-day feeding followed by transfer of the larvae to untreated semisynthetic diet, larval weight after 10 days was only a fraction of that in the controls at all concentrations between 0.05% and 0.006% (Table 7).

Table 7

Effects of different concentrations of azadirachtin on the development of *Ostrinia nubilalis* (30 larvae per concentration; larval survival and average weight were determined 10 days after the start of the experiment).

Azadirachtin concentration (%)	Treated seedlings			Untreated seedlings		
	Survival of larvae (%)	Average weight/larva (mg±SE)	Pupation [and rate of emergence] (%)	Survival of larvae (%)	Average weight/larva (mg±SE)	Pupation [and rate of emergence] (%)
0.05	53	2.8±0.5*	0 [0]	60	39.2±5.1	57 [54]
0.025	40	9.1±2.4*	0 [0]	77	61.0±4.4	60 [60]
0.01	57	10.5±3.3*	3 [0]	60	39.2±5.1	57 [54]
0.0075	63	6.5±0.4*	0 [0]	68	21.8±2.7	52 [48]
0.006	56	8.4±1.0*	4 [4]	68	21.8±2.7	52 [48]
0.005	70	25.5±3.4*	27 [27]	77	61.0±4.4	60 [60]

\*Significantly different from untreated plants at P=0.05 (Duncan's Multiple Range Test).

Only 27% of the larvae pupated in the 0.005% treatment, 4% in the 0.006% treatment and none, or practically none, in treatments with higher concentrations. In addition, the residual effect of 0.025% azadirachtin was investigated 2, 4 and 6 days after treatment (Table 8), as had been done with NSKE.

Table 8

Residual effect of 0.025% azadirachtin sprayed on corn seedlings on the development of Ostrinia nubilalis (30 larvae per day; larval survival and average weight were determined 10 days after the start of the experiment).

Infestation of plants, days after treatment	Treated seedlings			Untreated seedlings		
	Survival of larvae (%)	Average weight/ larva (mg±SE)	Pupation [and rate of emergence] (%)	Survival of larvae (%)	Average weight/ larva (mg±SE)	Pupation [and rate of emergence] (%)
2	56	6.5±0.8*	0 [0]	56	53.5±4.7	51 [51]
4	28	28.7±8.7*	11 [8]	57	57.8±4.1	57 [53]
6	44	15.6±4.7*	16 [16]	72	50.0±3.9	52 [48]

\* Significantly different from untreated plants in the corresponding control at P = 0.05 (Duncan's Multiple Range Test).

The larval weight 10 days after the start of exposure to treated seedlings was significantly lower than on untreated ones. No pupae developed from larvae fed on treated seedlings 2 days after treatment, and of those fed on seedlings with 4- and 6-day-old residues - only 11% and 16%, respectively, pupated.

Similar work was conducted with another corn pest, the corn borer Sesamia nonagrioides (Melamed-Madjar et al., 1989). Exposure for 3 days to fresh, 0-day-old aqueous NSKE residues on corn seedlings (Table 9) showed good activity at the 1%, 0.5% and 0.25% concentrations (zero, zero and 10% pupation, respectively); activity ceased at 0.1%.

Table 9

Effects of fresh, 0-day-old residues obtained from different spray concentrations of an aqueous NSKE on the development of Sesamia nonagrioides (30 larvae per experiment; larval survival and average weight were determined 13 days after the start of the experiment).

NSKE concentration (%)	Treated plants			Untreated plants		
	Survival of larvae (%)	Average weight/ larva (mg±S.E.)	Pupa-tion (%)	Survival of larvae (%)	Average weight/ larva (mg±S.E.)	Pupa-tion (%)
1.0	0	-	0	63.3	82.0 <sup>†</sup> ±10.7	50
0.5	30	7.2±1.8*	0	77	49.9±2.4	66
0.25	40	28.3±5.7*	10	60	93.7±6.9	60
0.1	70	61.9±9.8*	43.3	56	134.4±12.8	50
0.05	83	52.3±4.2*	60	73	101.9 <sup>†</sup> ±7.0	53

\* Significantly different from untreated plants at P=0.01 (Duncan's Multiple Range Test).



If the NSKE concentrations effective in this test on Sesamia (Table 9) are compared with those effective on Ostrinia (Table 4), it becomes evident that S. nonagrioides was less susceptible to neem extract than was the European corn borer. The residual effect of 1% aqueous NSKE on corn plants was investigated after 1, 3 and 7 days (Table 10), as had been done for O. nubilalis.

Table 10

Residual effect of 1% aqueous NSKE, sprayed on corn seedlings, on the development of Sesamia nonagrioides larvae (30 larvae per experiment; larval survival and average weight were determined 13 days after the start of the experiment).

Infestation of plants, days after treatment	Treated plants			Untreated plants		
	Survival of larvae (%)	Average weight/ larva (mg±S.E.)	Pupation (%)	Survival of larvae (%)	Average weight/ larva (mg±S.E.)	Pupation (%)
1	53	15.8±4.6*	10	73.3	50.6±8.7	67
3	40	25.6±4.5**	10	88	58.1±7.1	67
7	50	39.2±7.3	36.9	70	58.9±7.1	53

Significantly different from untreated plants at \*P = 0.01 and \*\*P = 0.05 (Duncan's Multiple Range Test).

The average larval weight 13 days after the start of the experiment was significantly lower on the treated plants infested 1 or 3 days after the treatment, than on the control plants (Table 10). The percent pupation of larvae exposed to 1- and 3-day-old neem residues was only 10%, whereas with 7-day-old residues the pupation rate approached that of the control.

Another insect investigated in our laboratory at the Volcani Center was a serpentine leafminer, Liriomyza trifolii (Meisner et al., 1986b). For this investigation we used a series of NSKEs, most of which had been tried on S. littoralis (see above), viz., aqueous, methanol, ethanol, acetone, isopropanol, n-butane, chloroform, carbon tetrachloride and n-pentane extracts. The extracts were either painted or sprayed on bean (Phaseolus vulgaris) seedlings and assayed against preimaginal stages of L. trifolii in two test situations: either (i) preinfestation application of neem, that is, either painting or spraying the leaves before exposure to ovipositing females; or (ii) postinfestation application of neem, viz., treatment after the appearance of the first mines following exposure to ovipositing females. Among all extracts the ethanol and methanol extracts showed on painting the highest activity in both pre- and postinfestation application. With preinfestation application by spraying, the aqueous, methanol and ethanol extracts were active, whereas only the methanol extract showed good activity in postinfestation spray application.

By drenching the soil, in which bean seedlings had been grown, with the aqueous extract, systemic properties against Liriomyza were demonstrated (Table 11).

Table 11

The systemic effect on the pupal yield of Liriomyza trifolii, obtained upon soil treatment with a 1% aqueous neem extract.

Infested on indicated days after soil treatment	Total number of pupae/plant		% Reduction in pupal yield
	Treatment ± S.E.	Control ± S.E.	
1	5.6±1.9*	21.8±4.0	74.4
3	4.7±4.1*	22.5±3.3	79.2
5	1.4±3.1*	30.4±4.8	95.4

\*Significantly different from control (solvent-only treated leaves) at P = 0.05 (Duncan's Multiple Range Test).

The best results - 95% reduction in pupal yield - were obtained when the plants were exposed to ovipositing females 5 days after the soil treatment. The results were less striking when the leaves were exposed to the ovipositing females 1 and 3 days after treatment, namely, respective reductions of 74% and 79% in pupal yield.

In view of the locust calamities which hit certain areas in Africa in recent years, it might be of interest to consider the experiments conducted by the author during a sabbatical spent with Prof. G.H. Schmidt at the University of Hannover, FRG. We investigated the antifeedant effect of methanolic NSKE and azadirachtin on Eyprepocnemis plorans, a locust species found in the Mediterranean area, especially in maritime regions. Acridids belonging to different species and subfamilies exhibit extremely marked differences in their response to neem components. For instance, adults of the desert locust, S. gregaria, refused to feed on corn leaves treated with neem kernel suspensions at concentrations 50 times lower than those needed to obtain the same effect with adults of the migratory locust, Locusta migratoria (Pradhan and Jotwani, 1971). Even more striking were findings (Mulkern and Mongolkiti, 1975) that numerous American grasshopper species were highly tolerant against the antifeedant effects of azadirachtin. Fourth-instar E. plorans nymphs were therefore offered either methanolic NSKE- or azadirachtin-treated saccharose-impregnated filter paper disks or treated leaves of broad bean (Vicia faba). The amount of substrate consumed was determined by weighing the filter paper or measuring the leaf area before and after exposure to the nymphs (Ascher et al., 1989). On filter paper (Table 12), both NSKE<sub>-4</sub> and azadirachtin were considerably active down to the 10<sup>-4</sup> treatment.

Table 12

Antifeedant effect of methanolic NSKE and azadirachtin (AZA) offered to fourth-instar Eyprepocnemis plorans nymphs on saccharose-coated filter paper.

Concn of treatment solution (%)		Cumulative weight of filter paper consumed per pair of nymphs (mg, mean $\pm$ S.E.)		
		2 days	4 days	6 days
Saccharose filter paper (control)		6.5 $\pm$ 0.8 ac	19.8 $\pm$ 2.0 ag	33.1 $\pm$ 2.7 a
NSKE	10 <sup>-2</sup>	1.0 $\pm$ 0.9 bde	1.5 $\pm$ 1.2 bc	1.6 $\pm$ 1.1 bd
	10 <sup>-3</sup>	0.4 $\pm$ 0.4 bd	1.6 $\pm$ 1.6 bc	2.8 $\pm$ 2.6 bd
	10 <sup>-4</sup>	0.4 $\pm$ 0.3 bd	2.0 $\pm$ 1.2 bc	3.2 $\pm$ 1.6 bd
	10 <sup>-5</sup>	4.3 $\pm$ 1.2 af	14.3 $\pm$ 2.9 adf	23.3 $\pm$ 3.8 c
	10 <sup>-6</sup>	7.7 $\pm$ 1.1 c	14.8 $\pm$ 1.7 df	22.4 $\pm$ 3.6 cf
AZA	10 <sup>-3</sup>	0.2 $\pm$ 0.1 b	0.4 $\pm$ 0.2 b	0.4 $\pm$ 0.2 b
	10 <sup>-4</sup>	2.2 $\pm$ 0.9 d	5.0 $\pm$ 2.1 ce	7.8 $\pm$ 3.6 de
	10 <sup>-5</sup>	3.1 $\pm$ 1.2 ef	8.8 $\pm$ 3.1 ef	14.7 $\pm$ 4.0 ef
	10 <sup>-6</sup>	8.4 $\pm$ 1.7 cg	23.2 $\pm$ 2.9 g	36.8 $\pm$ 4.2 a
	10 <sup>-7</sup>	10.8 $\pm$ 1.4 g	23.4 $\pm$ 2.3 g	48.2 $\pm$ 4.8 g
	10 <sup>-9</sup>	7.9 $\pm$ 1.4 c	26.4 $\pm$ 4.9 g	50.7 $\pm$ 6.3 g

Within columns, figures followed by a common letter do not differ significantly at  $P = 0.05$  (Duncan's Multiple Range Test).

In the leaf treatment (Table 13), however, azadirachtin was definitely more active than NSKE, with 100% deterrence at as low as 10<sup>-4</sup>% with azadirachtin and only 10<sup>-2</sup>% with NSKE.

Table 13

Antifeedant effect of methanolic NSKE, AZA, and 'Neemark' offered to fourth-instar Eyprepocnemis plorans nymphs on broad bean leaves; results recorded after 6 days.

Concn of treatment solution (%)		Leaf area consumed per pair of nymphs (cm <sup>2</sup> , mean $\pm$ S.E.)
Untreated leaves (control)		20.6 $\pm$ 1.9 a
NSKE	10 <sup>-1</sup>	0 b
	7x 10 <sup>-2</sup>	0 b
	3x 10 <sup>-2</sup>	0 b
	10 <sup>-2</sup>	0 b
	2x 10 <sup>-3</sup>	2.1 $\pm$ 1.1 b
'Neemark'	2x 10 <sup>-3</sup>	9.4 $\pm$ 2.0 c
NSKE	10 <sup>-3</sup>	14.7 $\pm$ 2.0 d
AZA	10 <sup>-2</sup>	0 b
	10 <sup>-3</sup>	0 b
	10 <sup>-4</sup>	0 b
	10 <sup>-5</sup>	3.1 $\pm$ 1.9 b
	10 <sup>-6</sup>	11.6 $\pm$ 2.2 cd
	10 <sup>-7</sup>	21.8 $\pm$ 1.7 a

Figures followed by a common letter do not differ significantly at P = 0.05 (Duncan's Multiple Range Test).

The methanolic NSKE was somewhat more active than the commercial Indian neem preparation 'Neemark'.

Finally, I would like to report on some work on spider mites, namely, on NSKEs from various solvents on bean leaves repelling and reducing the fecundity of the carmine spider mite, Tetranychus cinnabarinus (Mansour and Ascher, 1983). On the basis of EC-50s (in parentheses) for repellency, the order of effectiveness of the extracts was pentane (0.04%) > chloroform (0.05%) > n-butanol (0.14) > acetone (0.2%) > methanol (0.37) > the water extract, which was inactive; the same order of activity held true for reduction of fecundity: pentane (0.05%) > chloroform (0.08%) > n-butanol (0.11%) > acetone (0.17%) >> methanol (1.0%). The activity thus decreased with increasing dielectric constant of the extracting solvent; that is, just the contrary to the situation in insects, e.g. S. littoralis or L. trifolii (see above). Since the chloroform and butanol extracts in the original solvents were phytotoxic, 7-day-old residues on bean leaves were investigated with the pentane and acetone extracts only. Residues thus aged for 7 days caused mortality, and reduction in fecundity of adult females, but no repellency. The acetone and the pentane extracts sprayed directly on adult female mites on bean leaf disks, caused repellency and reduction of fecundity and also mortality of adults.

It was subsequently deemed of interest to compare the effects of the NSKEs from different solvents on the phytophagous mite, with those on the predacious mite Phytoseiulus persimilis (Mansour et al., 1987). All the extracts were much more toxic (as judged by mortality incurred) to the pest arthropod than to its predator (Table 14).

Table 14

Percent mortality ( $\pm$  S.E.) in females of Tetranychus cinnabarinus and Phytoseiulus persimilis engendered by 1-h-old residues of neem extracts from different solvents on bean leaves.

Concentration (%)	Extracts of			
	Methanol	Ethanol	Acetone	Pentane
<u>Phytoseiulus persimilis</u> <sup>z</sup>				
Control (solvents only)	0	0	0	6 $\pm$ 3.0
0.05	0 b <sup>y</sup>	0 b	0 b	25 $\pm$ 3.5 a
0.1	12 $\pm$ 2.5 b	0 c	0 c	35 $\pm$ 3.5 a
0.2	20 $\pm$ 3.5 a	0 b	0 b	20 $\pm$ 3.5 a
0.3	22 $\pm$ 2.5 a	0 b	0 b	27 $\pm$ 3.4 a
0.5	41 $\pm$ 3.3 a	0 c	21 $\pm$ 4.3 b	27 $\pm$ 4.4 b
1.0	45 $\pm$ 3.5 a	14 $\pm$ 3.7 c	31 $\pm$ 4.3 b	30 $\pm$ 3.2 b
2.0	45 $\pm$ 5.7 a	65 $\pm$ 9.7 a	58 $\pm$ 4.6 a	45 $\pm$ 5.7 a
4.0	50 $\pm$ 6.5 a	66 $\pm$ 5.3 a	58 $\pm$ 5.2 a	57 $\pm$ 7.2 a
8.0	65 $\pm$ 5.7 a	82 $\pm$ 6.4 a	76 $\pm$ 5.8 a	76 $\pm$ 5.3 a
LC-50 (%)	2.4	2.5	2.3	3.5
95% C.I.	(1.4-5.2)	(0.8-9.0)	(1.3-4.9)	(1.5-31.5)
Slope ( $\pm$ S.E.)	0.8 $\pm$ 0.12	2.3 $\pm$ 0.61	1.5 $\pm$ 0.28	0.7 $\pm$ 0.19
<u>Tetranychus cinnabarinus</u> <sup>x</sup>				
Control	0	0	0	7 $\pm$ 2.5
0.05	6 $\pm$ 2.9 b	10 $\pm$ 3.5 b	48 $\pm$ 5.2 a	50 $\pm$ 5.7 a
0.1	14 $\pm$ 1.9 c	22 $\pm$ 4.1 c	57 $\pm$ 6.0 b	70 $\pm$ 3.5 a
0.2	30 $\pm$ 6.1 c	32 $\pm$ 4.6 c	63 $\pm$ 7.2 b	92 $\pm$ 4.1 a
0.3	37 $\pm$ 3.7 c	40 $\pm$ 7.3 c	74 $\pm$ 3.7 b	96 $\pm$ 1.9 a
0.5	40 $\pm$ 7.1 c	43 $\pm$ 5.4 c	80 $\pm$ 3.5 b	100 a
1.0	58 $\pm$ 4.6 c	62 $\pm$ 6.1 c	82 $\pm$ 4.6 b	100 a
2.0	60 $\pm$ 3.5 c	74 $\pm$ 5.3 b	100 a	100 a
4.0	97 $\pm$ 2.0 a	98 $\pm$ 1.2 a	100 a	100 a
LC-50 (%)	0.8	0.6	0.1	0.06
95% C.I.	(0.5-1.3)	(0.4-0.8)	(0.03-0.15)	(0.05-0.07)
Slope ( $\pm$ S.E.)	1.5 $\pm$ 0.24	1.5 $\pm$ 0.17	1.1 $\pm$ 0.19	2.5 $\pm$ 0.27

<sup>z</sup>100-200 predacious mites were used for each concentration, in 6 to 20 replicates.

<sup>y</sup>Within rows, means followed by the same letter are not significantly different at  $P = 0.05$  (Duncan's Multiple Range Test).

<sup>x</sup>100 mites were used for each concentration, in five replicates.



Using the respective LC-50s for the two species, the toxicity index - LC-50 *Phytoseiulus* divided by LC-50 *Tetranychus* - was calculated from the data in Table 14 and found to be 3 for the methanol, 4 for the ethanol, 23 for the acetone, and 58 for the pentane extract. The order of toxicity of the extracts for *Phytoseiulus* was methanol = ethanol = acetone > pentane extract, and thus different from that for *Tetranychus*, which was pentane > acetone > ethanol > methanol. In other words, the pentane extract, which was the most toxic to *T. cinnabarinus*, was the least toxic extract to *P. persimilis*. However, the reduction in fecundity in *P. persimilis* was nearly equal to that of *T. cinnabarinus* (Table 15).

Table 15  
Percent reduction in fecundity ( $\pm$ S.E.) in females of *Tetranychus cinnabarinus* and *Phytoseiulus persimilis* engendered by 1-h-old residues of neem extracts from different solvents on bean leaves.

Concentration (%)	Extracts of			
	Methanol	Ethanol	Acetone	Pentane
<i>Phytoseiulus persimilis</i> <sup>z</sup>				
0.05	0 b <sup>y</sup>	0 b	9 $\pm$ 1.9 a	12 $\pm$ 2.5 a
0.1	69 $\pm$ 1.9 a	9 $\pm$ 1.9 b	65 $\pm$ 3.5 a	15 $\pm$ 3.5 a
0.2	75 $\pm$ 1.6 a	25 $\pm$ 2.2 d	62 $\pm$ 2.5 b	43 $\pm$ 2.5 c
0.3	82 $\pm$ 3.4 a	32 $\pm$ 2.5 c	62 $\pm$ 3.4 b	86 $\pm$ 4.3 a
0.5	81 $\pm$ 3.3 a	46 $\pm$ 2.9 c	68 $\pm$ 2.6 b	80 $\pm$ 3.5 a
1.0	82 $\pm$ 3.4 a	76 $\pm$ 2.9 a	85 $\pm$ 3.5 a	84 $\pm$ 5.8 a
2.0	79 $\pm$ 3.3 a	72 $\pm$ 2.5 a	83 $\pm$ 3.4 a	77 $\pm$ 5.2 a
4.0	92 $\pm$ 2.5 b	97 $\pm$ 2.0 ab	92 $\pm$ 2.5 b	100
8.0	91 $\pm$ 3.3 b	99 $\pm$ 1.0 a	96 $\pm$ 2.9 ab	100
EC-50 (%)	0.1	0.6	0.15	0.2
95% C.I.	(0.0-0.3)	(0.4-0.7)	(0.04-0.3)	(0.06-0.5)
Slope ( $\pm$ S.E.)	0.9 $\pm$ 0.31	1.8 $\pm$ 0.18	1.0 $\pm$ 0.21	1.6 $\pm$ 0.39
<i>Tetranychus cinnabarinus</i> <sup>z</sup>				
0.05	10 $\pm$ 1.6 b	31 $\pm$ 2.9 a	34 $\pm$ 3.4 a	31 $\pm$ 4.3 a
0.1	18 $\pm$ 2.5 b	31 $\pm$ 4.3 b	54 $\pm$ 10.4 a	52 $\pm$ 7.2 a
0.2	22 $\pm$ 4.6 b	35 $\pm$ 5.7 b	64 $\pm$ 3.7 a	80 $\pm$ 7.1 a
0.3	34 $\pm$ 5.3 b	47 $\pm$ 6.0 b	82 $\pm$ 6.4 a	86 $\pm$ 2.9 a
0.5	35 $\pm$ 5.0 b	50 $\pm$ 7.1 b	89 $\pm$ 5.1 a	97 $\pm$ 2.0 a
1.0	50 $\pm$ 3.5 c	72 $\pm$ 4.1 b	94 $\pm$ 2.9 a	94 $\pm$ 2.9 a
2.0	97 $\pm$ 2.0 a	97 $\pm$ 2.0 a	100 a	100 a
4.0	100 a	100 a	100 a	100 a
EC-50 (%)	0.5	0.3	0.09	0.09
95% C.I.	(0.28-0.98)	(0.13-0.47)	(0.08-0.1)	(0.05-0.12)
Slope ( $\pm$ S.E.)	1.7 $\pm$ 0.32	1.3 $\pm$ 0.25	1.6 $\pm$ 0.14	1.9 $\pm$ 0.25

<sup>z</sup>For both species 60-100 mites were used for each concentration, in six to ten replicates.

<sup>y</sup>Within rows, means followed by a common letter are not significantly different at P = 0.05 (Duncan's Multiple Range Test).

From the EC-50s for the reduction of fecundity in Table 15, the fecundity index (EC-50 of Phytoseiulus divided by EC-50 Tetranychus) was found to be 1.7 for acetone, 2.0 for ethanol and 2.2 for pentane, whereas P. persimilis was more susceptible than T. cinnabarinus to the methanol extract, as regards reduction of fecundity.

In this context one should consider our results (Mansour et al., 1986) on the toxicity of neem for a spider, Chiracanthium mildei (Table 16).

Table 16

Mortality of Chiracanthium mildei exposed for 48 h to residues of 4% neem extracts on grapefruit leaves (six spiders/replicate; four replicates/test; 1 h after treatment).

Dried neem extract prepared with	% Mortality $\pm$ S.D. after		
	2 days <sup>*</sup>	5 days <sup>*</sup>	10 days <sup>*</sup>
Pentane	8 $\pm$ 1	54 $\pm$ 10	71 $\pm$ 9
Acetone	0	12 $\pm$ 8	54 $\pm$ 9
Ethanol	0	8 $\pm$ 2	33 $\pm$ 5
Methanol	0	0	4 $\pm$ 1
Water	0	0	4 $\pm$ 1
Control (acetone only)	0	0	4 $\pm$ 1

\* Includes 48 h of exposure time.

The order of toxicity of the residues from 4% extracts from the following solvents, 2, 5 and 10 days after the start of a 2-day exposure, was: pentane > acetone > ethanol >> methanol = water (non-toxic).

All the extracts at 2.5% were nontoxic 10 days after the start of exposure (Table 17).

Table 17

Mortality of Chiracanthium mildei 8 days after a 48-h exposure to residues of neem extracts at different concentrations on grapefruit leaves (six spiders/replicate; four replicates/test; 1 h after treatment).

Dried neem extract prepared with	% Mortality $\pm$ S.D. on concentration (%)			
	2.5	4	6	8
Pentane	0	66 $\pm$ 9	78 $\pm$ 8	89 $\pm$ 6
Acetone	0	50 $\pm$ 8	60 $\pm$ 7	80 $\pm$ 8
Ethanol	0	30 $\pm$ 5	45 $\pm$ 4	55 $\pm$ 4
Methanol	0	4 $\pm$ 1	22 $\pm$ 2	44 $\pm$ 5
Water	0	4 $\pm$ 1	4 $\pm$ 1	4 $\pm$ 1
Control (acetone only)	4 $\pm$ 1	4 $\pm$ 1	4 $\pm$ 1	4 $\pm$ 1

Neem is a classical example of so-called recent technological innovations that have been with us, though little known, for many years. It is a long-established Indian insecticide and a folk remedy (since ancient times) mentioned in earliest Sanskrit medical writings and used in Ayurvedic medicine. Neem, however, became a really exciting subject in pest control only in the 1960s and '70s, following the findings of Pradhan et al. (1962, 1963) in India, and of Leuschner (1972), a graduate student of Prof. Schmutterer, in Africa. This proves again that 'chance favors only the prepared mind'.

## REFERENCES

- Ascher, K.R.S. 1981. Some physical (solubility) properties and biological (sterilant for Epilachna varivestis females) effects of a dried methanolic Neem (Azadirachta indica) seed kernel extract. p. 63-74. In H. Schmutterer, K.R.S. Ascher, and H. Rembold (eds), Natural Pesticides from the Neem Tree (Azadirachta indica A. Juss), Proc. 1st Int. Neem Conf., Rottach-Egern, FRG, 16-18 June 1980. GTZ, Eschborn, FRG.
- Ascher, K.R.S. 1987. Notes on Indian and Persian lilac pesticide research in Israel. p. 45-53. In H. Schmutterer and K.R.S. Ascher (eds), Natural Pesticides from the Neem Tree and Other Tropical Plants, Proc. 3rd Int. Neem Conf., Nairobi, Kenya, 10-15 July 1986. GTZ, Eschborn, FRG.
- Ascher, K.R.S., M. Eliyahu, N.E. Nemny, and J. Meisner. 1984. Neem seed kernel extract as an inhibitor of growth and fecundity in Spodoptera littoralis. p. 331-344. In H. Schmutterer and K.R.S. Ascher (eds), Natural Pesticides from the Neem Tree and Other Tropical Plants. Proc. 2nd Int. Neem Conf., Rauischholzhausen, FRG, 25-28 May 1983. GTZ, Eschborn, FRG.
- Ascher, K.R.S. and R. Gsell. 1981. The effect of neem seed kernel extract on Epilachna varivestis Muls. larvae. Zeitschr. Pflanzenkrankh. Pflanzenschutz 88:764-767.
- Ascher, K.R.S., M. Streloke, G.H. Schmidt, and J.D. Warthen, Jr. 1989. The antifeedant effect of neem seed kernel extract and azadirachtin on nymphs of Eyprepocnemis plorans. Phytoparasitica 17: 167-174.
- Jacobson, M. 1987. The neem tree: natural resistance par excellence. ACS Symp. Ser. 29: 220-232.
- Jacobson, M. (ed) 1989. Focus on Phytochemical Pesticides. Vol. I. The Neem Tree. CRC Press, Boca Raton, FL.
- Lavie, D., M.K. Jain, and S.R. Shpan-Gabrielith. 1967. A locust phagorepellent from two Melia species. Chem. Commun: 910-911.
- Leuschner, K. 1972. Effect of an unknown plant substance on a shield bug. Naturwissenschaften 59: 217-218.
- Mansour, F.A. and K.R.S. Ascher. 1983. Effects of neem (Azadirachta indica) seed kernel extracts from different solvents on the carmine spider mite, Tetranychus cinnabarinus. Phytoparasitica 11: 177-185.

Mansour, F.A., K.R.S. Ascher, and N. Omari. 1986. Toxicity of neem (Azadirachta indica) seed kernel extracts prepared with different solvents, on the spider Chiracanthium mildei. Phytoparasitica 14: 73-76.

Mansour, F., K.R.S. Ascher, and N. Omari. 1987. Effects of neem (Azadirachta indica) seed kernel extracts from different solvents on the predacious mite Phytoseiulus persimilis and the phytophagous mite Tetranychus cinnabarinus. Phytoparasitica 15: 125-130.

Meisner, J. and K.R.S. Ascher. 1984. Insect growth-regulating (IGR) effects of neem products on Spodoptera littoralis. p. 345-352. In H. Schmutterer and K.R.S. Ascher (eds), Natural Pesticides from the Neem Tree and Other Tropical Plants, Proc. 2nd Int. Neem Conf., Rauischholzhausen, FRG, 25-28 May 1983. GTZ, Eschborn, FRG.

Meisner, J., K.R.S. Ascher, and R. Aly. 1981. The residual effect of some products of neem seeds on larvae of Spodoptera littoralis in laboratory and field trials. p. 157-170. In H. Schmutterer, K.R.S. Ascher, and H. Rembold (eds), Natural Pesticides from the Neem Tree (Azadirachta indica A. Juss), Proc. 1st Int. Neem Conf., Rottach-Egern, FRG, 16-18 June 1980. GTZ, Eschborn, FRG.

Meisner, J., K.R.S. Ascher, and M. Zur. 1983. The residual effect of a neem seed kernel extract sprayed on fodder beet against larvae of Spodoptera littoralis. Phytoparasitica 11: 51-54.

Meisner, J., M. Kehat, M. Zur, and C. Eizick. 1978. Response of Earias insulana Boisd. larvae to neem (Azadirachta indica A. Juss) kernel extract. Phytoparasitica 6: 85-88.

Meisner, J., V. Melamed-Madjar, K.R.S. Ascher, and S. Tam. 1985. Effect of an aqueous extract of neem seed kernel on larvae of the European corn borer, Ostrinia nubilalis. Phytoparasitica 13: 173-178.

Meisner, J., V. Melamed-Madjar, S. Tam, and K.R.S. Ascher. 1986a. The effect of azadirachtin on larvae of the European corn borer, Ostrinia nubilalis. Zeitschr. Pflanzenkrankh. Pflanzenschutz. 93: 585-589.

Meisner, J., M. Wysoki, and K.R.S. Ascher. 1976. The residual effect of some products from Neem (Azadirachta indica A. Juss) seeds upon larvae of Borania (Ascotis) selenaria Schiff. in laboratory trials. Phytoparasitica 4: 185-192.

Meisner, J., S. Yathom, S. Tal, and K.R.S. Ascher. 1986b. The effect of various extracts of neem seed kernel on Liriomyza trifolii (Burgess) (Diptera: Agromyzidae). Zeitschr. Pflanzenkrankh. Pflanzenschutz 93: 146-152.

Melamed-Madjar, V., J. Meisner, and K.R.S. Ascher. 1989. Effect of neem on the corn borer Sesamia nonagrioides Lefebvre (Lepidoptera: Noctuidae). Zeitschr. Pflanzenkrankh. Pflanzenschutz 96: 521-525.

Mulkern, G.B. and S. Mongolkiti. 1975. Desert locust feeding deterrent ineffective against North American grasshoppers (Orthoptera Acrididae). Acrida 4: 95-103.

- Pradhan, S. and M.G. Jotwani. 1971. Neem kernel as antifeedant for locust. *Sneha Sandesh* 13: 1-5.
- Pradhan, S., M.G. Jotwani, and B.K. Rai. 1962. The neem seed deterrent to locust. *Indian Fmg* 12(8): 7,11.
- Pradhan, S., M.G. Jotwani, and B.K. Rai. 1963. The repellent properties of some neem products. *Bull. Reg. Res. Lab. Jammu* 1: 149-151.
- Saxena, R.C. 1989. Insecticides from neem. *ACS Symp. Ser.* 387: 110-135.
- Schmutterer, H. 1987. Insect growth-disrupting and fecundity-reducing ingredients from the neem and chinaberry trees. p. 119-170. *In* E.D. Morgan and N.B. Mandava (eds), *CRC Handbook of Natural Pesticides*. Vol. III (Part B). *Insect Growth Regulators*. CRC Press, Boca Raton, FL.
- Schmutterer, H. 1988. Potential of azadirachtin-containing pesticides for integrated pest control in developing and industrialized countries. *J. Insect Physiol.* 34: 713-719.
- Schmutterer, H. 1990. Properties and potential of natural pesticides from the neem tree, Azadirachta indica. *Annu. Rev. Ent.* 35: 271-297.
- Schmutterer, H. and K.R.S. Ascher. (eds). 1987. *Natural Pesticides from the Neem Tree and Other Tropical Plants*. *Proc. 3rd Int. Neem Conf., Nairobi, Kenya, 10-15 July 1986*. GTZ, Eschborn, FRG.



## ABSTRACT

Neem, Margosan-O, was used on potato cv. Russet Burbank against pyrethroid resistant (resistance ratios > 1000 - adult) Colorado potato beetle, *Leptinotarsa decemlineata* (Say), in field trials at three locations, Rosemount, Minn., Crookston, Minn., and Grand Forks, N.D. Sprays of 20 ppm azadirachtin were applied weekly, 3 times per generation, using tractor-drawn high pressure hydraulic sprayers. Sprays were applied against both first and second generation Colorado potato beetle larvae at Rosemount and against first generation only at Crookston and Grand Forks. When Colorado potato beetle larvae hatched on neem-treated foliage there was essentially no survival, but Colorado potato beetle larvae established prior to treatment were not adequately controlled. Tank mixing neem with a conventional insecticide for the first application greatly improved Colorado potato beetle control as measured by counts of beetle larvae and plant defoliation. Yields were taken only at Rosemount. Yield losses in all neem treatments were higher than that which occurred in the best conventional insecticide treatment primarily because neem did not control potato leafhopper, *Empoasca fabae* (Harris). However, beetle control and yields in neem-treated plots were comparable or better than obtained in plots treated with Colorado potato beetle specific *Bacillus thuringiensis* (Berliner) insecticides.

## INTRODUCTION

Pyrethroid insecticides were introduced to potato production in the Red River Valley of Minnesota and North Dakota in 1981. Almost immediately, Pydrin® (*fenvalerate*) became the insecticide of choice for control of Colorado potato beetle, *Leptinotarsa decemlineata* (Say). Although in the late 1970s and early 1980s Colorado potato beetle populations seldom exceeded the economic threshold, most growers sprayed beetles at least once and many treated more often. Resulting control was outstanding, but each year beetle pressure appeared to increase. In 1985, a grower near Karlstad, Minn. reported suspected resistance to Ambush® (*permethrin*).

In 1982, Johnston (1984) tested pyrethroid-naïve beetles from Glyndon, Minn., and determined the LD<sub>50</sub> for Pydrin to be 0.17 µg/adult beetle. In 1985, Watrin (1986) tested beetle populations from various Minnesota and North Dakota locations for pyrethroid resistance. LD<sub>50</sub>s ranged from 5.1 to 15.9 µg/adult beetle for Pydrin and from 3.5-10.2 µg for Ambush, except the problem population at Karlstad which had an LD<sub>50</sub> of 31.9. Resistance levels have increased each year since (Radcliffe and Lagnaoui 1989a) and in 1989 adult beetles from most Minnesota and North Dakota locations were virtually immune (resistance ratios > 1,000) to topical applications of Pydrin, Asana® (*esfenvalerate*) and Ambush (unpublished data).

Colorado potato beetle larvae are susceptible to neem-based insecticides (Steets 1976). Sprayed weekly, 2% ethanolic extracts of neem seed kernel gave better than 80% control of Colorado potato beetle larvae in Indiana field trials (Reed and Reed 1986), control equivalent to that obtained with Monitor® (*methamidophos*). Cantelo (cited in Zehnder and Warthen 1988) reported that 1% neem extract effectively controlled Colorado potato beetle but was severely phytotoxic to 'Kennebec' potatoes. Zehnder and Warthen showed that ingestion of 0.4-1.2% neem extracts resulted in significant mortality of Colorado potato beetle larvae within 72 h. Control was improved by the addition of the synergist piperonyl butoxide (PBO). Adult mortality was not significant but feeding was reduced. In the field, all rates of neem resulted

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E. B. Radcliffe is a professor and A. Lagnaoui a Ph.D. student in the Department of Entomology, University of Minnesota, St. Paul, MN 55108.

in significant yield increases of 'Superior' potatoes compared to the untreated control, but all neem treatments yielded less adjacent plots treated with permethrin. Although phytotoxicity was not evident, results suggested that repeated applications of neem extract or high rates of neem, especially in combination with PBO, may adversely affect yields.

Schmutterer (1986) has shown that exposure to residues of neem kernel extract for as little as 24 h greatly reduces fecundity of Colorado potato beetle and that some females become sterile. This effect apparently is irreversible. The suggestion was made that under field conditions one or two sprays at the beginning of the reproductive period might provide season-long control.

The experiments reported here were designed to test the efficacy of neem against populations of Colorado potato beetle from three locations, Rosemount, Crookston and Grand Forks. There is little commercial potato production in the vicinity of Rosemount and beetles from this location are characterized by an intermediate level of pyrethroid resistance. Grand Forks and Crookston are in the heart of Red River Valley potato growing region and beetles at both locations are highly resistant to pyrethroids. In preliminary studies at Rosemount (Radcliffe and Lagnaoui 1989b), a commercial neem product, Margosan-O, gave excellent control of Colorado potato beetle when sprayed at a concentration of 20 ppm azadirachtin (1:150 Margosan-O/water) in 935 l water/ha. In laboratory trials (unpublished data) we observed 100% mortality of Colorado potato beetle larvae hatching from eggs masses on potato foliage dipped in a 5 ppm concentration of azadirachtin. Here we report trials in which Margosan-O was compared with other possible alternative Colorado potato beetle insecticides such as *Bacillus thuringiensis* (Berliner), Dowco-473 (an insect growth regulator), and Kryocide® (cryolite), and with two standard potato insecticides, the pyrethroid Asana® and the organophosphate Guthion® (*azinhosphomethyl*).

## MATERIALS AND METHODS

Potatoes, cv. Russet Burbank were planted 18 May at the University of Minnesota Agricultural Experiment Station, Rosemount, Minn., and 10 May at the Red River Valley Potato Grower's Association Research Farm, Grand Forks, N.D. and the University of Minnesota Northwest Experiment Station, Crookston, Minn. Plots were 8 rows x 16 m with 1.02 m row spacing and replicated 4 times at Rosemount, and 6 rows x 13 m with 0.97 m row spacing and replicated 3 times at Crookston and Grand Forks. Completely randomized plot designs were used at all 3 locations.

Treatments consisted of different insecticidal sprays used alone, tank-mixed or applied sequentially. At each location, insecticidal sprays were applied weekly, three times against first generation Colorado potato beetle larvae. At Rosemount, three additional applications were made against second generation potato beetle larvae.

At Rosemount, spray treatments used were: 1) three applications of ABG-6263 (*Bacillus thuringiensis*, var. *tenebrionis*) at 1.12 kg formulated product/ha; 2) three applications of ABG-6263 at 2.24 kg Al/ha; 3) three applications of Asana at 0.028 kg Al/ha; 4) Asana at 0.028 kg/ha followed by two applications of M-One® (*Bacillus thuringiensis* var. *san diego*) at 4.67 l/ha; 5) Dowco-473 at 0.035 kg Al/ha + Guthion at 0.56 kg Al/ha tank-mixed followed by two applications of Dowco-473 at 0.035 kg Al/ha; 6) rotation of Guthion at 0.56 kg Al/ha, M-One at 4.67 l/ha, and Kryocide (*cryolite*) at 12.33 kg formulated product/ha; 7) three applications of Margosan-O at 0.018.7 kg azadirachtin/ha (20 ppm); 8) Margosan-O at 0.0187 kg Al/ha + Asana at 0.028 kg/ha tank-mixed followed by 2 applications of Margosan-O at 0.0187 kg Al/ha; 9) Margosan-O at 0.0187 kg Al/ha + Guthion at 0.56 kg Al/ha followed by two applications of Margosan-O at 0.0187 kg Al/ha.; 10) M-One at 4.67 l/ha + Guthion at 0.56 kg Al/ha followed by two applications of M-One at 4.67 l/ha; 11) three applications of Trident® (*Bacillus thuringiensis* var. *tenebrionis*) at 9.35 l/ha; 12) Trident at 9.35 l/ha + Guthion at 0.56 kg Al/ha tank-mixed followed by 2 applications of Trident at 9.35 l/ha; 13) untreated control.

At Crookston, spray treatments used were: 1) three applications of Asana at 0.028 kg AI/ha; 2) Asana at 0.028 kg/ha followed by two applications of M-One at 4.67 l/ha; 3) three applications of Guthion at 0.56 kg AI/ha; 4) three applications of Margosan-O at 0.015 kg AI/ha (20 ppm); 5) Margosan-O at 0.015 kg AI/ha + Guthion at 0.56 kg AI/ha tank-mixed followed by two applications of Margosan-O at 0.015 kg AI/ha; 6) M-One at 4.67 l/ha + Guthion at 0.56 kg AI/ha followed by two applications of M-One at 4.67 l/ha; 7) three applications of Trident at 9.35 l/ha; 8) Trident at 9.35 l/ha + Asana at 0.028 kg AI/ha tank-mixed followed by 2 applications of Trident at 9.35 l/ha; 9) untreated control.

At Grand Forks, spray treatments used were: 1) three applications of Asana at 0.028 kg AI/ha; 2) Asana at 0.028 kg/ha followed by two applications of M-One at 4.67 l/ha; 3) Dowco-473 at 0.035 kg AI/ha + Guthion at 0.56 kg AI/ha tank-mixed followed by two applications of Dowco-473 at 0.035 kg AI/ha; 4) three applications of Guthion at 0.56 kg AI/ha; 5) rotation of Guthion at 0.56 kg AI/ha, M-One at 4.67 l/ha, and Kryocide at 12.33 kg/ha; 6) three applications of Margosan-O at 0.0049 kg AI/ha (20 ppm); 7) Margosan-O at 0.0049 kg AI/ha + Asana at 0.028 kg/ha tank-mixed followed by 2 applications of Margosan-O at 4.9 kg AI/ha; 8) Margosan-O at 0.0049 kg AI/ha concentration + Guthion at 0.56 kg AI/ha followed by two applications of Margosan-O at 0.0049 kg AI/ha; 9) M-One at 4.67 l/ha + Guthion at 0.56 kg AI/ha followed by two applications of M-One at 4.67 l/ha; 10) three applications of Trident at 9.35 l/ha; 11) Trident at 9.35 l/ha + Guthion at 0.56 kg AI/ha tank-mixed followed by 2 applications of Trident at 9.35 l/ha; 12) untreated control.

Insecticidal sprays were applied using tractor-drawn, high pressure, boom sprayers. At Rosemount and Crookston, the sprayer was equipped with drop nozzles, three hollow-cone nozzles to the row. At Grand Forks, the sprayer had a single Teejet® nozzle over each row. At Rosemount, sprays were applied in 935 l water/ha at 1035 kPa on 28 June, 8 July and 15 July against first generation Colorado potato beetle larvae and on 30 July, 7 August and 15 August against second generation Colorado potato beetle larvae. At Crookston, sprays were applied in 748 l water/ha at 620 kPa on 28 June, 5 July and 12 July. At Grand Forks, sprays were applied in 243 l water/ha at 275 kPa on 30 June, 5 July and 12 July. Margosan-O was sprayed at the same concentration (1:150 dilution) at each location; thus the rate of azadirachtin applied varied with spray volume, 0.0187 kg/ha at Rosemount, 0.015 kg/ha at Crookston, and 0.0049 kg/ha at Grand Forks.

Colorado potato beetle larvae were counted on 10 randomly selected plants from each of the two middle rows of each plot. Larvae were recorded by size class, instars I and II were classified as small, instars III and IV as large. At Rosemount, first generation Colorado potato beetle larvae were counted 30 June, 10 and 17 July, and second generation Colorado potato beetle were counted 2, 9 and 16 August. At Crookston and Grand Forks, first generation Colorado potato larvae were counted 29 June (prespray, representative plots only), 4, 11 and 21 July. Visual estimates of percent defoliation in each plot were made at the end of each larval generation by 2-5 persons on each sampling date. At Rosemount, plots were scored for defoliation 17 July and 16 August. At Crookston and Grand Forks, plots were scored for defoliation 12 July and 21 July. At Rosemount, potato leafhopper nymphs were counted on 35 leaves per plot 17 July, 2, 9 and 16 August. At Rosemount, tuber yields were estimated by harvesting 2 rows from the middle of each plot 25 September. Analysis of variance was used to test treatment means for significant differences in Colorado potato beetle larvae, potato leafhopper nymphs, percent defoliation and tuber yield. Tuber yields for the Rosemount experiment were regressed on plot means of Colorado potato beetle larvae per 20 plants, percent defoliation, potato leafhopper nymphs per 35 leaves, and percent defoliation plus potato leafhopper per 35 leaves. Means used in regression analyses were per sampling date with untreated controls excluded. Analyses of variance were run using the Statistical Analysis System (SAS Institute 1988), and means were separated ( $P < 0.05$ ) by Duncan's multiple range test. MULTREG was used to run regressions (Weisberg 1986).



Hatch of first generation Colorado potato beetle eggs began about 25 June and was largely completed by 4 July at all three locations. Maximum daily temperatures 1-10 July at Rosemount ranged from 31-35°C, at Crookston from 27-37°C, and at Grand Forks from 30-36°C, resulting in rapid larval development. Larvae at Rosemount and Crookston were approximately an instar ahead of those at Grand Forks. Many larvae were already in the second and third instars when the first insecticide applications were made. For neem and the *B.t.* insecticides our first application was already too late for optimum Colorado potato beetle control. Peak larval populations at Rosemount occurred between the sampling dates of 30 June and 10 July, whereas at Crookston and Grand Forks peak larval populations occurred about the 4 July sampling date (Figures 1-3).

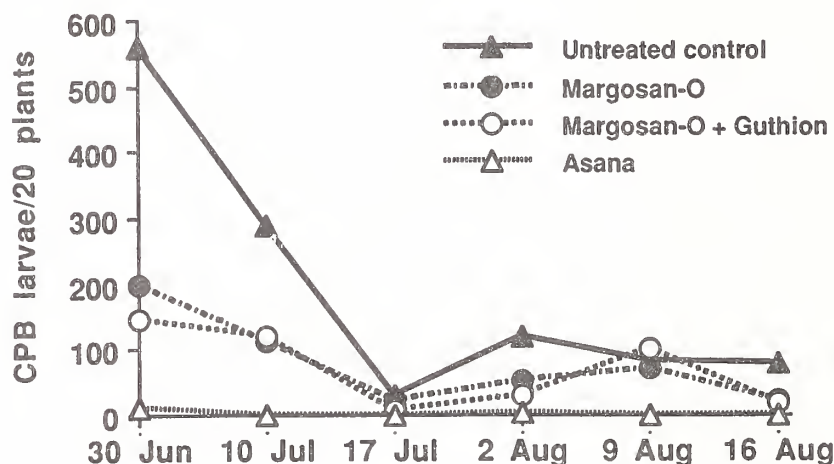


Figure 1. Numbers of Colorado potato beetle larvae on plots of selected insecticidal treatments, Rosemount, Minn., 1989.

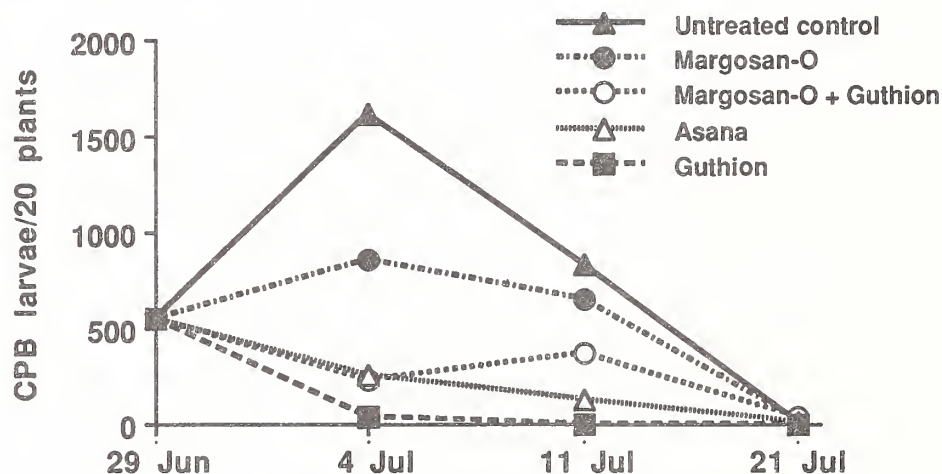


Figure 2. Numbers of Colorado potato beetle larvae on plots of selected insecticidal treatments, Crookston, Minn., 1989.

All insecticides were more efficacious in suppressing Colorado potato beetle larvae and beetle caused defoliation injury at Rosemount than at Crookston or Grand Forks (Table 1). These differences were probably due to better spray coverage resulting from the greater volume of water and higher spray pressure used at Rosemount. Control was generally poorest at Grand Forks where sprays were applied in the least water and at the lowest pressure. Water volumes were particularly critical for the Margosan-O because

rates of azadirachtin applied varied accordingly. However, growers in the Red River Valley typically apply insecticides in 10-15 gal of water per acre so it is doubtful they could be converted to high volume applications. Higher spray concentrations were not used out of concern for possible phytotoxicity.

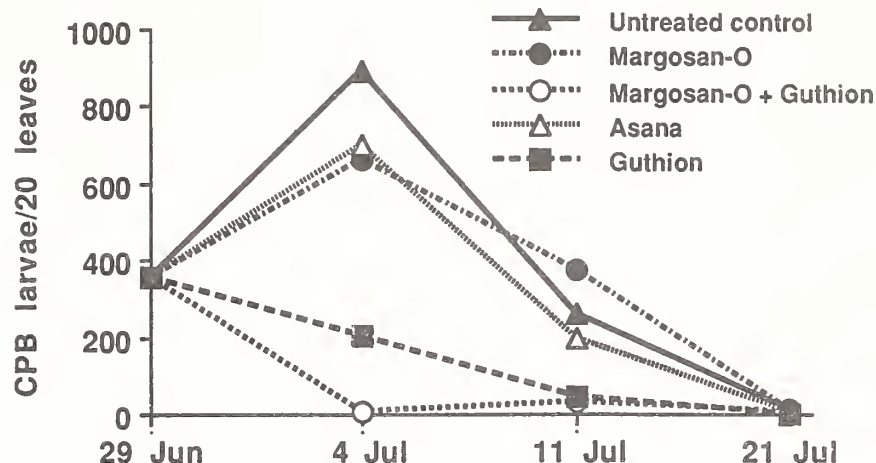


Figure 3. Numbers of Colorado potato beetle larvae on plots of selected insecticidal treatments, Grand Forks, N.D., 1989.

Table 1. Control of pyrethroid resistant Colorado potato beetle with neem and other insecticides at three locations in Minnesota and North Dakota, 1989.

Treatment <sup>1/</sup>	Appl. <sup>2/</sup>	Rate(s) <sup>3/</sup>	CPB <sup>4/</sup>	Defol. <sup>5/</sup>	PLH <sup>6/</sup>	Yield <sup>7/</sup>
<i>Rosemount</i>						
ABG-6263	3	1.12 kg AI/ha	62.8cd	6.9bc	93.8d	21.48d-f
ABG-6263	3	2.24 kg AI/ha	45.2bc	7.0c	95.8de	19.81ef
Asana	3	0.028 kg AI/ha	5.6a	1.3a	0.4a	35.91a
Asana	1	0.028 kg/ha	4.1a	4.1a-c	2.9a	27.83b
+ M-One	2	4.67 l/ha				
Dowco-473 + Guthion	1	0.035 kg AI/ha + 0.56 kg AI/ha	4.2a	2.1a	7.4a	27.38bc
+ Dowco-473	2	0.035 kg AI/ha				
Guthion	1	0.56 kg AI/ha	29.3ab	5.1a-c	7.3a	23.32de
+ M-One	1	4.67 l/ha				
+ Kryocide	1	12.33 kg/ha				
Margosan-O	3	0.0187 kg AI (azadirachtin)/ha	81.1d	5.4a-c	82.2c	23.25de
Margosan-O + Asana	1	0.0187 kg AI/ha + 0.028 kg AI/ha	7.1a	2.6ab	7.8a	28.09b
+ Margosan-O	2	0.0187 kg AI/ha				
Margosan-O + Guthion	1	0.0187 kg AI/ha + 0.56 kg/ha	69.8cd	5.5a-c	16.2b	23.10de
+ Margosan-O	2	0.0187 kg AI/ha				
M-One + Guthion	1	4.68 l/ha + 0.56 kg AI/ha	30.1ab	3.5a-c	18.9b	23.75cd
+ M-One	2	4.68 l/ha				
Trident	3	9.35 l/ha	48.8bc	7.1c	95.3de	18.90f



Table 1 (Continued).

Treatment <sup>1/</sup>	Appl. <sup>2/</sup>	Rate(s) <sup>3/</sup>	CPB <sup>4/</sup>	Defol. <sup>5/</sup>	PLH <sup>6/</sup>	Yield <sup>7/</sup>
Trident + Asana	1	9.35 l/ha + 0.56 kg Al/ha	1.6a	2.0a	4.2a	28.79b
+ Trident	2	9.35 l/ha				
Untreated control			167.9e	56.4d	102.1e	9.73g
<i>Crookston</i>						
Asana	3	0.028 kg Al/ha	134.0a	9.6a		
Asana	1	0.028 kg Al/ha	212.8ab	7.5a		
+ M-One	2	4.67 l/ha				
Guthion	3	0.56 kg Al/ha	13.2a	1.4a		
Margosan-O	3	0.015 kg Al/ha	513.7c	59.2cd		
Margosan-O + Guthion	1	0.015 kg Al/ha + 0.028 kg Al/ha	212.3ab	14.0a		
+ Margosan-O	2	0.015 kg Al/ha				
M-One + Guthion	1	4.68 l/ha + 0.56 kg Al/ha	89.1a	7.5a		
+ M-One	2	4.68 l/ha				
Trident	3	9.35 l/ha	481.8bc	45.8bc		
Trident + Asana	1	9.35 l/ha + 0.028 kg Al/ha	243.0ab	27.0ab		
+ Trident	2	9.35 l/ha				
Untreated control			821.5d	79.0d		
<i>Grand Forks</i>						
Asana	3	0.028 kg Al/ha	302.8b-d	36.3ab		
Asana	1	0.028 kg Al/ha	190.1a-c	22.1ab		
+ M-One	2	4.67 l/ha				
Dowco-473 + Guthion	1	0.035 kg Al/ha + 0.56 kg Al/ha	9.8a	6.9a		
+ Dowco-473	2	0.035 kg Al/ha				
Guthion	3	0.56 kg Al/ha	84.1	6.3a		
Guthion	1	0.56 kg Al/ha	126.6ab	27.8a		
+ M-One	1	4.67 l/ha				
+ Kryocide	1	12.33 kg/ha				
Margosan-O	3	0.0049 kg Al/ha	350.0cd	56.5b		
Margosan-O + Asana	1	0.0049 kg Al/ha + 0.028 kg Al/ha	185.6a-c	22.8ab		
+ Margosan-O	2	0.0049 kg Al/ha				
Margosan-O + Guthion	1	0.0049 kg Al/ha + 0.56 kg Al/ha	15.2a	3.9a		
+ Margosan-O	2	0.56 kg Al/ha				
M-One + Guthion	1	4.68 l/ha + 0.56 kg Al/ha	52.2a	6.1a		
+ M-One	2	4.68 l/ha				
Trident	3	9.35 l/ha	291.1b-d	28.3ab		

Table 1 (Continued).

Treatment <sup>1/</sup>	Appl. <sup>2/</sup>	Rate(s) <sup>3/</sup>	CPB <sup>4/</sup>	Defol. <sup>5/</sup>	PLH <sup>6/</sup>	Yield <sup>7/</sup>
Trident + Guthion	1	9.35 l/ha + 0.56 kg AI/ha	120.2ab	13.6a		
+ Trident	2	9.35 l/ha				
Untreated control			388.4d	51.9b		

<sup>1/</sup>Insecticidal sprays were applied three times against first generation Colorado potato beetle larvae. At Rosemount, three additional applications were made against second generation larvae.

<sup>2/</sup>At Rosemount, sprays were applied in 935 l water/ha 28 June, 8, 15, 30 July, 7 and 15 August. At Crookston, sprays were applied in 748 l water/ha at 620 kPa 28 June, 5 July and 12 July. At Grand Forks, sprays were applied in 243 l water/ha at 275 kPa 30 June, 5 July and 12 July.

<sup>3/</sup>Margosan-O was applied at a spray concentration of 20 ppm azadirachtin (0.0187 kg AI/ha at Rosemount, 0.015 kg AI/ha at Crookston and 0.0049 kg AI/ha at Grand Forks).

<sup>4/</sup>Mean number of Colorado potato beetle per 20 plants per sampling date. At Rosemount, Colorado potato larvae were counted 30 June, 10 and 17 July, and 2, 9 and 16 August. At Crookston and Grand Forks, larvae were counted 29 June, 4, 11 and 21 July.

<sup>5/</sup>Mean percent defoliation per sampling date. At Rosemount, plots were scored for defoliation 17 July and 16 August. At Crookston and Grand Forks, plots were scored for defoliation 12 July and 21 July.

<sup>6/</sup>Mean number of potato leafhopper nymphs per 35 leaves per plot per sampling date. Potato leafhopper nymphs counted 17 July, 2, 9 and 16 August.

<sup>7/</sup>Tuber yields were estimated by harvesting 2 rows from the middle of each plot 25 September.

Means flanked by the same letter within a column are not significantly different ( $P > 0.05$ ; Duncan's multiple range test [SAS Institute 1988]).

At Rosemount, all insecticidal treatments kept mean defoliation per sampling date in the range of 1.3-7.1%. Mean defoliation in the untreated check was 56.4%. Asana alone or in combination with other insecticides gave the best control of Colorado potato beetle larvae. Margosan-O alone reduced Colorado potato beetle larvae numbers only 58%, but protected plants sustained just 5.4% defoliation. Tank-mixing Margosan-O with Asana significantly improved control of Colorado potato beetle larvae, decreased defoliation and increased tuber yield over that obtained with Margosan-O alone, but tank-mixing with Guthion was of less benefit. Similarly, application of Trident + Asana was more effective than M-One + Guthion. Guthion has been the primary potato insecticide used at Rosemount for the past several years and these results suggest that resistance to Guthion is developing there. Control of Colorado potato beetle, reduction of defoliation, and tuber yields obtained with Margosan-O or Margosan-O plus conventional insecticide combinations were essentially equal to that obtained with the various *B.t.* treatments. Margosan-O and the *B.t.* insecticides gave no control of potato leafhopper nymphs.

At Crookston and Grand Forks beetle pressure was greater than at Rosemount and the plants were under severe moisture stress. Under these conditions there was little compensatory growth of new foliage. When the first generation of summer beetles emerged the last week of July, all plots were totally defoliated within 24 h terminating these experiments. Guthion and combinations with Guthion were the most effective treatments at both Red River Valley locations. Across all sampling dates, Asana alone gave only 80% control of Colorado potato beetle larvae at Crookston and just 22% control at Grand Forks. Margosan-O alone provided almost no control of beetle larvae nor reduction of defoliation injury. However, when tank-mixed with Guthion for the first application, Margosan-O reduced both larvae and defoliation to levels not significantly different from that

obtained with three applications of Guthlon. At Grand Forks, the Margosan + Guthlon combination was actually the best treatment in terms of defoliation. This was remarkable since Guthlon was sprayed only once and the rates of azadirachtin used at Grand Forks were very low. Performance of the *B.t.* insecticides was similarly enhanced by tank-mixing with a conventional insecticide for the first application.

Tuber yields ( $y$ ) were correlated with mean number of Colorado potato beetle larvae ( $x$ ) per sampling date (Figure 4,  $y = 27.95 - 0.10x$ ,  $R^2 = 0.39$ , d.f. = 46, std. error of slope = 0.02). Correlation of tuber yields ( $y$ ) with large larvae only ( $x$ ) per sampling date was  $y = 27.87 - 0.17x$ ,  $R^2 = 0.39$ , d.f. = 46, std. error of slope = 0.02). Regression of tuber yields ( $y$ ) on small larvae only ( $x$ ) per sampling date was  $y = 27.76 - 0.23x$ ,  $R^2 = 0.36$ , d.f. = 46, std. error of slope = 0.01. Regression of tuber yields ( $y$ ) on first generation larvae only ( $x$ ) per sampling date was  $y = 27.32 - 0.07x$ ,  $R^2 = 0.35$ , d.f. = 46, std. error of slope = 0.02. Regression of tuber yields ( $y$ ) on second generation larvae only ( $x$ ) per sampling date was  $y = 26.81 - 0.08x$ ,  $R^2 = 0.21$ , d.f. = 46, std. error of slope = 0.01. First generation Colorado potato beetle were of greatest importance because hatch occurred when the plants were most vulnerable, at the onset of tuber-bulking. It is critical that control of first generation beetles be initiated in a timely manner to assure that early hatched larvae do not escape.

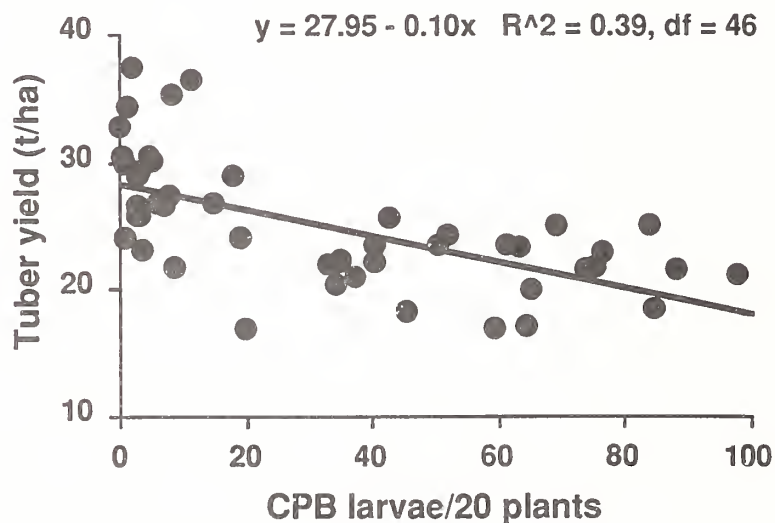


Figure 4. Relationship between tuber yield and mean number of Colorado potato beetle larvae per sampling date, Rosemount, Minn., 1989.

Tuber yields ( $y$ ) were correlated with mean percent defoliation ( $x$ ) per sampling date (Figure 5,  $y = 30.43 - 1.33x$ ,  $R^2 = 0.54$ , d.f. = 46, std. error of slope = 0.18). Yield was more highly correlated with defoliation than with numbers of beetle larvae because some treatments, e.g., Margosan-O and the *B.t.* insecticides, inhibit feeding but do not immediately kill the larvae. When tuber yields were regressed against mean number of potato leafhopper nymphs per sampling date the correlation was again high ( $y = 27.58 - 0.08x$ ,  $R^2 = 0.45$ , d.f. = 46, std. error of slope = 0.01). Use of both mean defoliation ( $x_1$ ) and mean number of potato leafhopper nymphs ( $x_2$ ) in the linear regression model yielded the best correlation ( $y = 30.36 - 0.93x_1 - 0.05x_2$ ,  $R^2 = 0.64$ , d.f. = 46, std. error of slope ( $x_1$ ) = 0.19, std. error of slope ( $x_2$ ) = 0.01.)

## CONCLUSIONS

In our experiments, neem sprays (Margosan-O) applied alone did not produce impressive reductions in numbers of Colorado potato beetle larvae. Larvae established and feeding before application on the foliage appeared to suffer little direct mortality. However, defoliation injury in neem-treated plots was disproportionately reduced, suggesting suppression of larval feeding. Tank-mixing neem with a conventional insecticide on first application

generally enhanced Colorado potato beetle control. Timing of application appeared to be critical. Colorado potato beetle larvae hatching on neem-treated foliage apparently sustained high mortality, and died without feeding. For neem to be effective without the addition of a conventional insecticide, first application would have to be made just before or almost immediately after first egg hatch. Margosan-O did not control potato leafhopper.

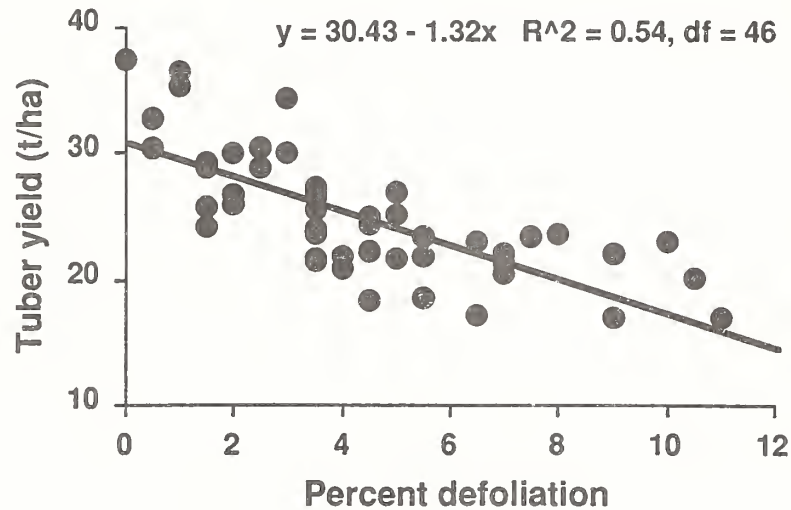


Figure 5. Relationship between tuber yield and mean percent defoliation by Colorado potato beetle per sampling date, Rosemount, Minn., 1989.

#### REFERENCES CITED

- Johnston, R. L. 1984. *Determination of Economic Thresholds for Potato Leafhopper, Empoasca fabae (Harris), and Action Thresholds for Colorado Potato Beetle, Leptinotarsa decemlineata (Say), on Potato in Minnesota*. Ph.D. Thesis, Univ. Minnesota. 82 pp.
- Radcliffe, E. B. and A. Lagnaoui, 1989a. Pyrethroid resistance in Colorado potato beetle: Minnesota-North Dakota. *Pesticide Resistance Newsletter* 1(2): 13-14.
- Radcliffe, E. B. and A. Lagnaoui, 1989b. Foliar sprays for control on potato, 1988. *Insect. Acaric. Tests* 14: 144-147.
- Reed, D. K. and G. L. Reed. 1986. Control of vegetable insects with neem seed extracts. *Proc. Indiana Acad. Sci.* 94: 335.
- SAS Institute. 1988. *SAS/STAT User's Guide, Release 6.03 Edition*. SAS Institute, Cary, N.C.
- Schmutterer, H. 1987. Fecundity-reducing and sterilizing effects of neem seed kernel extracts in the Colorado potato beetle, *Leptinotarsa decemlineata*, pp. 351-360, in: *Natural Pesticides from the Neem Tree (Azadirachta indica A. Juss) and Other Tropical Plants*, H. Schmutterer and K. R. S. Ascher, eds., Proc. 3rd Int. Neem Conf., Nairobi, Kenya, 1986, Schriftenreihe der GTZ, No. 206, Eschborn.

Steets, R. 1976. Zur Wirkung eines gereinigten Extraktes aus Früchten von *Azadirachta indica* S. Juss auf *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae). *Z. angew. Ent.* 82: 169-176.

Watrin, C. G. 1987. *Insecticide Resistance in Colorado Potato Beetle (Coleoptera: Chrysomelidae)*. M.S. Thesis, Univ. Minnesota. 39 pp.

Weisberg, S. 1986. *MULTREG User's Manual*. Univ. Minnesota, School of Statistics, Tech. Rep. No. 298R.

Zehnder, G. and J. D. Warthen. 1988. Feeding inhibition and mortality effects of neem-seed extract on the Colorado potato beetle (Coleoptera: Chrysomelidae). *J. Econ. Entomol.* 81: 1040-1044.



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ACTIVITY OF NEEM EXTRACT AND MARGOSAN-O FOR CONTROL OF COLORADO POTATO  
BEETLE IN VIRGINIA

G. W. Zehnder and J. W. Warthen

ABSTRACT

In laboratory experiments, concentrations of 0.4, 0.8, and 1.2% (wt/vol) neem seed extract inhibited feeding of larva and adult Colorado potato beetle (CPB), Leptinotarsa decemlineata (Say), on treated potato foliage. Significant larval mortality occurred 72 hours after feeding on treated foliage, however, adult mortality did not exceed 25% in any neem treatment. Toxicity and feeding inhibition effects of neem were enhanced by addition of the synergist piperonyl butoxide (PBO), suggesting that Virginia CPB may possess inherent resistance to neem. In field experiments, spray application of neem extract reduced numbers of CPB in treated potato plots, compared with control. The commercial formulation of neem, Margosan-O, was effective for control of CPB at concentration  $\geq 1.5\%$ . Tank mixes with PBO improved the efficacy of field applications of Margosan-O.

INTRODUCTION

Feeding damage by Colorado potato beetle (CPB), Leptinotarsa decemlineata (Say), is a primary factor limiting the production of white potatoes in eastern Virginia. Resistance of CPB to most classes of insecticides has been documented in Virginia and other potato-growing regions in the eastern United States (Forgash 1981). Lack of successful control of CPB with registered insecticides has generated interest in the testing of antifeedant compounds as crop protectants against CPB (Jermy et al. 1981, Hare et al. 1983). The antifeedant properties of seed from the neem tree, Azadirachta indica A. Juss, were first reported in field experiments with the adult desert locust (Pradhan & Jotwani 1968). The primary antifeedant component of neem seeds is azadirachtin, a triterpenoid first isolated by Butterworth & Morgan (1971) and characterized by Nakanishi (1975). Alternative structures have since been proposed by Kraus et al. (1985) and Broughton et al. (1986).

Previous efforts in the United States to evaluate neem against CPB indicated that a 1% concentration of neem extract protected 'Kennebec' potatoes from CPB attack (W. W. Cantelo, personal communication). However, phytotoxicity resulting from the neem formulation was severe. In 1985, preliminary field studies conducted by the authors in eastern Virginia indicated that CPB populations were significantly reduced in 'Superior' potatoes with no observed phytotoxicity following spray applications of 0.8% neem extract. Therefore, studies were continued in 1986 with eastern Virginia populations of CPB to evaluate feeding, mortality, and population trends following treatment with neem extract on potatoes. Additional field experiments were done in 1988 and 1989 to evaluate the effectiveness of Margosan-O (the commercial formulation of neem) for control of CPB.

MATERIALS AND METHODS

Laboratory Studies. A laboratory colony of CPB collected from the Eastern Shore Agricultural Experiment Station, Painter, Va., was established in May, 1986, on potato, Solanum tuberosum L. ('Red LaSoda'). Second instars and adults (7-10 d old) were collected from the colony and starved for 18 h before each experiment. Treatment dilutions were prepared from a viscous

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Zehnder is an entomologist, Virginia Polytechnic Institute and State University, Eastern Shore Agricultural Experiment Station, Painter, Va. 23420; Warthen is a research chemist, Insect Chemical Ecology Laboratory, Plant Sciences Institute, USDA-ARS, Beltsville, MD 20705.

concentrate of neem seed extract (Sample AI3-42845; Insect Chemical Ecology Laboratory, USDA-ARS, Beltsville, Md.) that contained 2.72 mg azadirachtin per gram of extract (Warthen et al. 1984). Specific amounts of extract were weighed to make 0.4, 0.8, and 1.2% (wt/vol) extract concentrations. Ethanol (2 ml) was added to dissolve the extract and 1 ml Triton X-100 (Rohm and Haas, Philadelphia) was used as an emulsifier. Sufficient water was added to obtain 1 liter of solution for each concentration. Heated water (32°C) resulted in a more uniform suspension than water at ambient temperature. The three neem concentrations were tested with and without the addition of the synergist piperonyl butoxide (PBO) at a ratio of 10:1 (PBO/azadirachtin).

Young potato leaves were removed from greenhouse-grown 'Red LaSoda' potatoes, submersed in the neem solutions for 5 s, and allowed to air-dry for 30 min. Control leaves were dipped in water containing ethanol and Triton X-100. Leaf disks (2.1 cm diameter) were cut from these leaves with a cork borer and placed on moist filter paper inside plastic Petri dishes (60 x 15 mm). Experiments were conducted separately for second instars and adults. The three neem concentrations, with and without PBO, were tested against each life stage. Two leaf disks per dish were used in the larval experiment and three leaf disks per dish were supplied for adults. In both experiments, replicates consisted of 2 CPB per dish, with 10 replicates per treatment. Petri dishes were placed in an environmental chamber maintained at 25°C, 65% RH, and a photoperiod of 16:8 (L:D). Treated leaf disks were replaced every 24 h for the duration of the test. Mortality of CPB in the Petri dishes and the leaf area of treated leaf disks were determined at 24-h intervals up to 72 h. Leaf disks (30) without CPB were placed in Petri dishes and kept in the environmental chamber during each 24-h test period. Mean leaf area of these undefoliated leaf disks was determined, and the remaining leaf area of the treatment leaf disks was subtracted from the undefoliated mean to obtain "leaf area consumed" values in each treatment. Leaf area was measured using a video camera mounted above an illuminated light box containing a digitizing unit (Skye Instruments, Buckingham, Pa.). The field of view was 13 x 13 cm with a resolution of approximately 0.5 mm. Leaf image was analyzed by a personal computer with Skye Image Analysis System software. Data were subjected to two-factor analysis of variance, and treatment means were compared by Duncan's Multiple Range test (SAS Institute 1985).

Field Studies. 'Superior' variety potatoes were planted in late March or early April at the Eastern Shore Agricultural Experiment Station, Painter, Va. in 1986, 1988, and 1989. Seed pieces were planted 0.3 m apart in plots consisting of three rows, (7.6 m long) and spaced 0.9 m apart. Treatment plots were replicated four times in a randomized complete block design. Neem extract or Margosan-0 solutions were applied weekly to the middle rows of each plot; two buffer rows bordered the treated rows. Applications were made with a compressed air backpack sprayer using three hollow-cone nozzles per row. Delivery rate was 700 liters/ha at 2.8 kg/cm<sup>2</sup> pressure.

CPB small larvae (first and second instars), large larvae (third and fourth instars), and adults were counted weekly by visual examination of ten randomly chosen stems per plot. Average percentage defoliation was calculated as the total percentage defoliation in each treatment divided by four (the number of replicate treatment rows). Potato tubers from all plants in the middle row of each treatment plot were dug after plant senescence. Grade 'A' tubers (min. diameter = 4.8 cm) were weighed to determine tuber yields in each treatment. Field data were analyzed with the same statistical procedures used for laboratory data.

## RESULTS

Laboratory Studies. More than 27% of larvae died during the first 24 h in the 0.8 and 1.2% neem + PBO treatments (Table 1). Data on leaf area consumed by larvae surviving after 24 h is not presented because sample numbers were too low for meaningful analysis. When data were subjected to

Duncan's analysis, leaf consumption values in all neem treatments were significantly less than control, except in the 0.8 and 1.2% concentrations without PBO. The addition of PBO to the neem extract resulted in significantly less feeding by larvae than in the neem treatments without PBO. In the neem treatments without PBO, mean leaf consumption values increased with increasing neem concentration, indicating a possible stimulatory effect of neem on larval feeding. This trend was not observed in the neem + PBO treatments, where values of leaf area consumed indicated an inverse relationship between feeding and neem concentration.

Percentage of mortality of larvae was significantly higher in the neem treatments than in control, and the addition of PBO resulted in significantly greater mortality at 24, 48, and 72 h (Table 1). Between 90 and 99% of larvae were dead 72 h after initiation of feeding in the three neem + PBO treatments.

Average potato leaf consumption values for adult CPB are presented in Table 2. Adult mortality did not exceed 25% after 72 h in any treatment, and differences in mean percentage of mortality between control and neem treatments were not significant ( $P > 0.05$ ). Adult feeding was significantly reduced in the neem treatments, and leaf consumption was further reduced in the PBO treatments.

Field Studies with Neem. Numbers of the predominant CPB life stages present in field plots on three sampling dates are presented in Table 3. Populations of all life stages were significantly lower in the 0.8 and 1.2% neem-treated plots than in control plots on most sampling dates. Plots sprayed with neem + PBO had lower numbers of small and large larvae on 26 May and adults on 2 June than in neem plots not treated with PBO. The addition of PBO did not result in significantly lower CPB numbers on earlier sample dates.

Differences in mean percent defoliation values corresponded to relative CPB densities in the various treatments (Table 4). Defoliation was significantly higher in the control plots than in neem-treated plots and the addition of PBO further reduced defoliation by CPB. Increasing neem concentration resulted in decreasing defoliation values in the neem and neem + PBO treatments.

Duncan's test analysis results indicated that mean tuber yield values in the neem-treated plots were significantly greater than in control plots, with the exception of the 1.2% neem + PBO treatment. The addition of PBO did not result in significantly greater yields than in neem plots without PBO. Despite low relative CPB populations and feeding damage, the 1.2% neem + PBO treatment resulted in the lowest tuber yield of all neem-treated plots. Adjacent potato plots receiving spray applications of permethrin (0.11 kg [AI]/ha) on the same dates as the neem applications produced tuber yields of 22.5 + 2.0 metric tons per ha, higher than yields in any neem treatment. These results suggest that, although phytotoxicity was not visually apparent, repeated applications of neem extract may have an adverse physiological effect on the plants. These effects may be more severe at higher neem concentrations in combination with PBO.

Field Studies with Margosan-0. During the 1988 test, Margosan-0 applied at concentrations of 0.2, 0.7, and 1.2% in water did not significantly ( $P > 0.05$ ) reduce numbers of CPB larvae or adults, compared with CPB density in untreated plots (data not presented). Percent defoliation on 17 June exceeded 90% in all Margosan-0 treatments, compared with 12% in plots sprayed with the pyrethroid insecticide esfenvalerate (Asana, Dupont De Nemours Co., Wilmington, De.) at recommended label rates.

In 1989, Margosan-0 was applied at concentrations of 1.0, 1.5, and 2.0%, with and without the synergist PBO. Numbers of large larvae were significantly ( $P < 0.05$ ) reduced in all Margosan-0 treatments, compared with control (Fig. 1). Larval populations were further reduced by the addition of PBO. Adult counts were more variable than larval numbers, but all concentrations of Margosan-0 in combination with PBO resulted in control of adults equivalent to the standard esfenvalerate treatment (Fig. 2).



Defoliation and tuber yield data (Figs. 3 and 4) indicated that Margosan-O concentrations  $\geq 1.5\%$  in combination with PBO resulted in effective control of CPB and commercially acceptable tuber yields. Unlike the crude neem extract, Margosan-O in combination with PBO had no apparent adverse effect on potato plant development.

## DISCUSSION

Researchers in Germany have demonstrated various adverse effects of neem extract against CPB larvae. These include inhibition of feeding (determined by relative weights of treated larvae) and postembryonic development, and direct mortality (Steets 1976, Schmutterer 1985). Percent mortality of fourth instars treated with neem was increased with addition of the synergist, PBO (Lange & Feuerhake 1984). The neem extract concentrations in these studies ranged from 0.025 to 0.1%, lower than the neem concentrations evaluated in the present study. Because azadirachtin contents of the German extracts were not determined, it is possible that the German extracts contained higher levels of azadirachtin and therefore exhibited greater activity against CPB than the neem extract used in our studies.

Results of the present study indicate that feeding (measured by actual leaf consumption) is significantly reduced in eastern Virginia CPB adults and larvae following neem application to potato foliage. Ingestion of 0.4-1.2% neem extract resulted in significant mortality of larvae 72 h after feeding was initiated, but adult mortality was not significant. Greater tolerance of adult CPB to various insecticides compared with larvae has been reported previously (Zehnder 1986). Because of significant larval mortality in the laboratory feeding experiments, it is not known whether reduced foliage consumption is caused by the antifeedant properties of neem or is a result of toxic effects that weakened the larvae and made them less able to feed. Adults feeding on neem-treated foliage consumed significantly less leaf material than adults in control treatments, but they exhibited no adverse physiological effects 72 h after feeding initiation. These results suggest that the most apparent effect of neem on adults exposed to treated foliage is one of avoidance or reduced acceptance, whereas the effect on larvae is one of decreased vigor or direct mortality.

Results of the field study demonstrate that neem extract and Margosan-O were effective under field conditions for control of CPB. However, the magnitude of the effects of neem on a field population of CPB will depend on which life stage is predominant during neem application. Because the laboratory experiments demonstrated greater toxic effects of neem against larvae than adults, field applications of neem for control of CPB would be most effective immediately after egg hatch, when small larvae are the predominant life stage.

Our results indicate that Margosan-O may be incorporated into a CPB management program, given its effectiveness. The increase in activity of Margosan-O against CPB with the addition of the mixed function oxidase enzyme inhibitor PBO suggests that Virginia CPB may possess inherent resistance to Margosan-O. This may be a result of cross resistance due to pre-exposure to another class of insecticide. However, the effectiveness of Margosan-O indicates that levels of resistance (at present) are not high. Infrequent use of Margosan-O early in the season against small larvae and in rotation with other insecticides will prolong the usefulness of Margosan-O and other materials.

A major factor limiting use of Margosan-O in potatoes may be cost. Approximately 2.5 quarts of Margosan-O is required to treat one acre of potatoes with a 1.5% concentration (in 40 gallons of water). At present, Virginia potato growers may not be willing to spend more than \$15.00 per acre per application of an insecticide. This situation may change depending on future effectiveness of registered insecticides against CPB and also the strength of the potato market in Virginia.

## References Cited

- Broughton, H. B., S. V. Ley, A. M. Z. Slawin, D. J. Williams, & E. D. Morgan. 1986. X-Ray crystallographic structure determination of detigloyldihydroazadirachtin and reassignment of the structure of the limonoid insect antifeedant azadirachtin. *J. Chem. Soc. Commun.* 1: 46-47.
- Butterworth, J. H. & E. D. Morgan. 1971. Investigation of the locust feeding inhibition of the seeds of the neem tree, *Azadirachta indica*. *J. Insect Physiol.* 17: 969-977.
- Forgash, A. J. 1981. Insecticide resistance of the Colorado potato beetle *Leptinotarsa decemlineata* (Say), pp. 34-46. In: J. H. Lashomb and R. Casagrande (eds.), *Advances in potato pest management*. Hutchinson Ross, Stroudsburg, Pa. 288 pp.
- Harcourt, D. G. 1964. Population dynamics of *Leptinotarsa decemlineata* (Say) in eastern Ontario. II. Population and mortality estimation during six age intervals. *Can. Entomol.* 96: 1190-1198.
- Hare, J. D., P. A. Logan, & R. J. Wright. 1983. Suppression of Colorado potato beetle, *Leptinotarsa decemlineata* (Say) populations with antifeedant fungicides. *Environ. Entomol.* 12: 1470-1477.
- Jermey, T., B. A. Butt, L. McDonough, D. L. Dreyer, & A. F. Rose. 1981. Antifeedants for the Colorado potato beetle. I. Antifeeding constituents of some plants from the sagebrush community. *Insect Sci. Appl.* 1: 237-242.
- Kraus, W., M. Bokel, A. Klenk, & H. Pohnl. 1985. The structure of azadirachtin and 22,23-dihydro-23B-methoxyazadirachtin. *Tetrahedron Lett.* 26: 6435-6438.
- Lange, V. W. & K. Feuerhake. 1984. Wirkungssteigerung von angereicherten Niemsamenextrakten durch den Synergisten Piperonylbutoxid unter Laborbedingungen. *Z. Angew. Entomol.* 98: 368-375.
- Nakanishi, K. 1975. Structure of the insect antifeedant azadirachtin. *Recent Adv. Phytochem.* 9: 238-298.
- Pradhan, S. & M. G. Jotwani. 1968. Neem as insect deterrent. *Chem. Age. (India)* 19: 756-759.
- SAS Institute. 1985. SAS user's guide. SAS Institute, Cary, N. C.
- Schmutterer, H. 1985. Which insect pests can be controlled by application of neem seed kernel extracts under field conditions? *Z. Angew. Entomol.* 100: 468-475.
- Steets, V. R. 1976. Zur Wirkung eines gereinigten Extraktes aus Früchten von *Azadirachta indica* A. Juss auf *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae). *Z. Angew. Entomol.* 82: 169-176.
- Warthen, Jr., J. D., J. B. Stokes & M. Jacobson. 1984. Estimation of azadirachtin content in neem extracts and formulations. *J. Liq. Chrom.* 7: 591-598.
- Zehnder, G. W. 1986. Timing of insecticides for control of Colorado potato beetle (Coleoptera: Chrysomelidae) in eastern Virginia based on differential susceptibility of life stages. *J. Econ. Entomol.* 79: 851-856.



Table 1

Effect of neem extract on feeding and mortality of second instar CPB

Concn (%)	Potato leaf area (cm <sup>2</sup> ) consumed per insect in 24 h ( $\pm$ SEM)	% Mortality ( $\pm$ SEM) <sup>a</sup>		
		24 h	48 h	72 h
Control	1.50 $\pm$ 0.08a	0.0 $\pm$ 0.0b	0.6 $\pm$ 0.6c	5.5 $\pm$ 2.9d
0.4	1.04 $\pm$ 0.14c	0.6 $\pm$ 0.6b	9.5 $\pm$ 1.6bc	65.5 $\pm$ 1.0bc
0.8	1.43 $\pm$ 0.11ab	0.6 $\pm$ 0.6b	2.5 $\pm$ 1.0c	50.0 $\pm$ 2.7c
1.2	1.65 $\pm$ 0.08a	0.6 $\pm$ 0.6b	34.5 $\pm$ 2.5b	72.7 $\pm$ 2.8bc
0.4 + PBO	1.16 $\pm$ 0.10bc	2.4 $\pm$ 1.0b	79.4 $\pm$ 4.3a	99.4 $\pm$ 0.6a
0.8 + PBO	0.89 $\pm$ 0.11cd	27.3 $\pm$ 4.1a	79.4 $\pm$ 3.0a	90.5 $\pm$ 3.0ab
1.2 + PBO	0.68 $\pm$ 0.12d	27.3 $\pm$ 1.4a	94.5 $\pm$ 1.4a	99.4 $\pm$ 0.6a

Means within columns followed by the same letter are not significantly different ( $P > 0.05$ ; Duncan's multiple range test [SAS Institute 1985]).

<sup>a</sup>Mean cumulative percent mortality.

Table 2

Effect of neem extract on feeding by CPB adults

Concn (%)	Potato leaf area (cm <sup>2</sup> ) consumed per insect per day ( $\pm$ SEM) at:		
	24 h	48 h	72 h
Control	5.51 $\pm$ 0.21a	5.00 $\pm$ 0.09a	5.80 $\pm$ 0.12a
0.4	4.68 $\pm$ 0.60ab	4.13 $\pm$ 0.51a	4.35 $\pm$ 0.67b
0.8	3.64 $\pm$ 0.74bc	1.74 $\pm$ 0.57b	1.45 $\pm$ 0.53c
1.2	2.08 $\pm$ 0.74c	1.66 $\pm$ 0.54b	0.92 $\pm$ 0.29cd
0.4 + PBO	4.57 $\pm$ 0.29ab	1.37 $\pm$ 0.32bc	0.55 $\pm$ 0.18cd
0.8 + PBO	3.57 $\pm$ 0.35bc	0.81 $\pm$ 0.24bc	1.58 $\pm$ 0.41c
1.2 + PBO	2.53 $\pm$ 0.31c	0.29 $\pm$ 0.15c	0.14 $\pm$ 0.03d

Means within columns followed by the same letter are not significantly different ( $P > 0.05$ ; Duncan's multiple range test [SAS Institute 1985]).

Table 3

Effectiveness of neem extract against field populations of CPB in potatoes, Painter, Va., 1986

Concn (%)	Mean no. CPB per stem		
	19 May	26 May	2 June
	SL	LL	Adults
Control	16.48a	12.23a	6.03a
0.4	5.63b	7.78b	2.41b
0.8	2.60bc	2.25c	1.35c
1.2	3.83bc	0.98cd	1.38c
0.4 + PBO	5.78b	2.08c	1.23c
0.8 + PBO	2.60bc	0.53d	0.85c
1.2 + PBO	2.03c	0.25d	0.95c

Means within columns followed by the same letter are not significantly different ( $P > 0.05$ ; Duncan's multiple range test [SAS Institute 1985]). SL, small larvae; LL, large larvae.

Table 4

Percent defoliation and tuber yield of 'Superior' potatoes following field application of neem extract, 1986

Concn (%)	Mean % defoliation ( $\pm$ SEM) on 9 June	Mean tuber yield ( $\pm$ SEM) (metric tons/ha)
Control	58.2 $\pm$ 9.1a	11.9 $\pm$ 2.4c
0.4	30.8 $\pm$ 6.6b	15.4 $\pm$ 1.5ab
0.8	13.2 $\pm$ 3.1c	15.1 $\pm$ 1.3ab
1.2	5.5 $\pm$ 2.4c	17.5 $\pm$ 2.6a
0.4 + PBO	7.2 $\pm$ 1.4c	16.3 $\pm$ 1.2ab
0.8 + PBO	3.7 $\pm$ 2.0c	16.3 $\pm$ 1.5ab
1.2 + PBO	2.8 $\pm$ 1.2c	13.8 $\pm$ 1.9bc

Means within columns followed by the same letter are not significantly different ( $P > 0.05$ ; Duncan's multiple range test [SAS Institute 1985]).

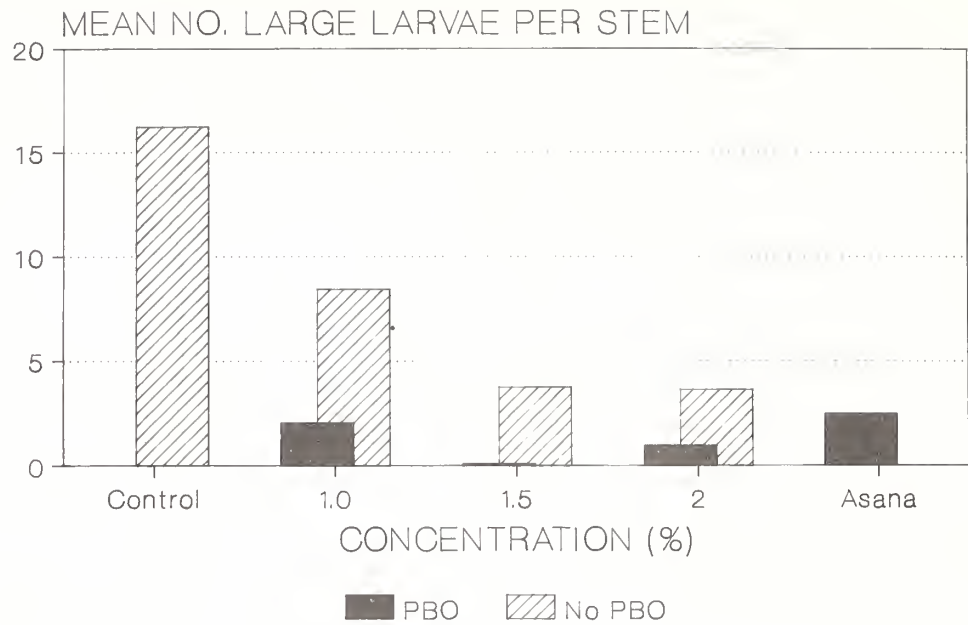


Figure 1  
Efficacy of 1-2% concentrations of Margosan-0 (with and without PBO) compared with Asana (esfenvalerate) for control of peak populations of CPB larvae, 1989.

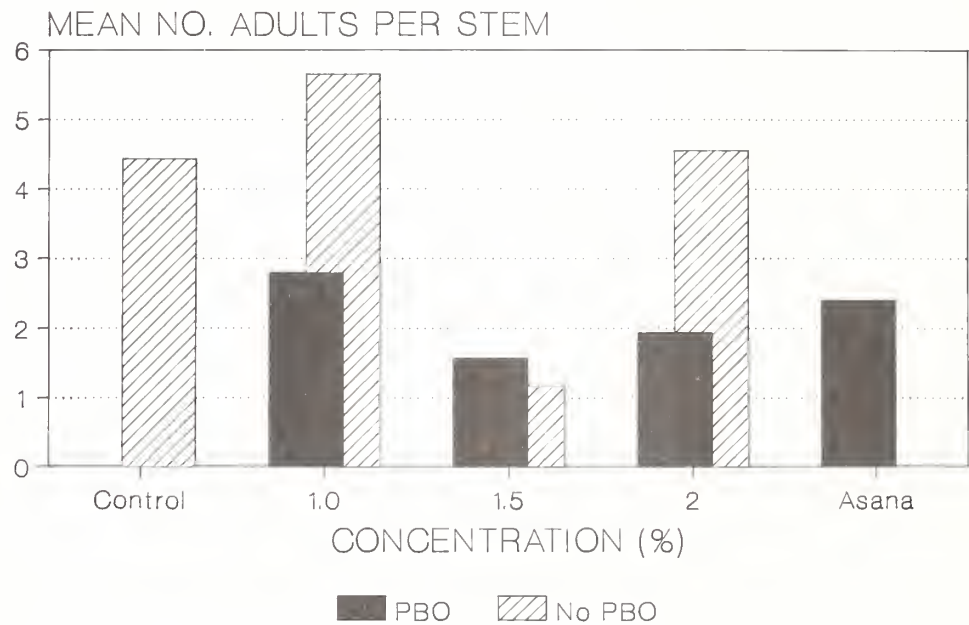


Figure 2  
Efficacy of 1-2% concentrations of Margosan-0 (with and without PBO) compared with Asana (esfenvalerate) for control of peak populations of CPB first generation adults, 1989.

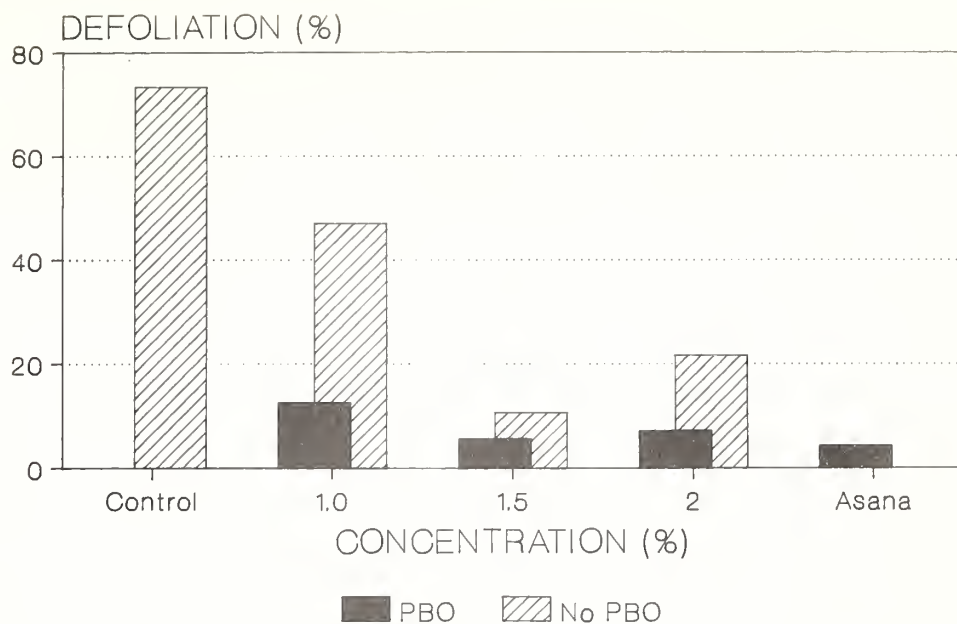


Figure 3  
Average percent defoliation in potato plots treated with Margosan-0 (with and without PBO) and Asana (esfenvalerate), 1989.

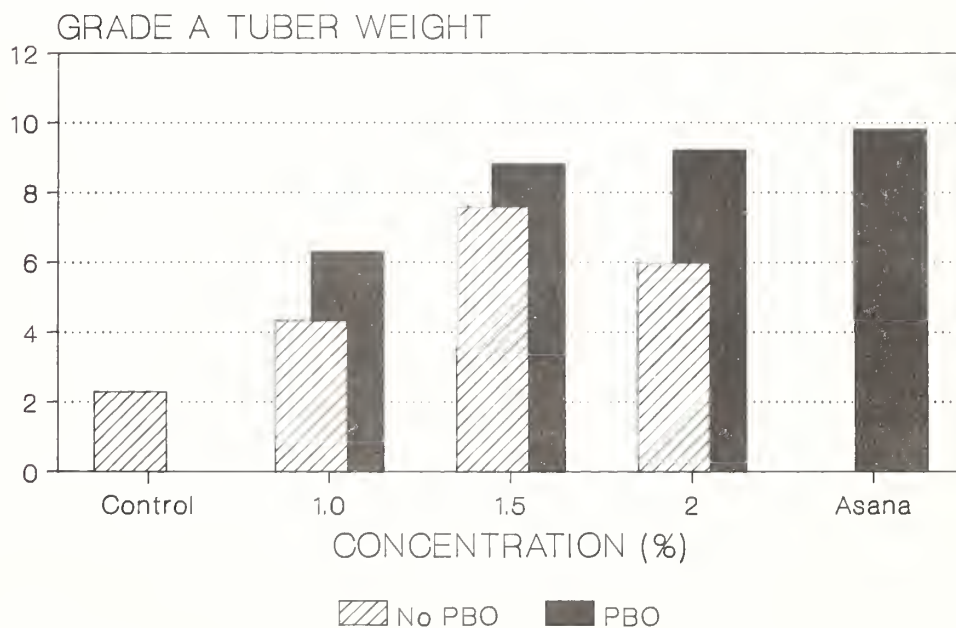


Figure 4  
Tuber yield (lbs grade A tubers per foot of row) in potato plots treated with Margosan-0 (with and without PBO) and Asana (esfenvalerate), 1989.

## Laboratory and Greenhouse Evaluations with Neem

### EFFICACY OF NEEM EXTRACTS AND NEEM DERIVATIVES AGAINST SEVERAL AGRICULTURAL INSECT PESTS

T. Wood

#### ABSTRACT

NPI is currently evaluating several insecticide formulations based on the chemistry of neem. This paper summarizes results from a number of laboratory bioassays designed to characterize the antifeedant and insect growth regulator (IGR) activities of neem extracts, azadirachtin and azadirachtin derivatives. Leaf disk and whole plant studies show feeding deterrence against fall armyworm, tobacco budworm and other insect pests, but questions remain concerning the commercial significance of this property. Moderate-to-high concentrations of azadirachtin are often required to deter feeding, and effects appear to be relatively short-lived in the field. The IGR activities of neem extracts and azadirachtin appear to be of greater commercial significance. Many economically important insect pests are affected, and efficacy is seen at commercially acceptable rates of application. The systemic activity of azadirachtin and the enhanced stability of hydrogenated azadirachtin contribute to the commercial potential of neem-based insecticides.

#### INTRODUCTION

For the past seven years, NPI has been working to develop commercial insecticides based on the chemistry of the neem tree, Azadirachta indica A. Juss. Initially, our program focused on the structure and activity of azadirachtin (AZA), the principal active constituent in extracts of neem fruits. During this phase of development, J. A. Klocke and coworkers concentrated their research on developing methods for isolating and purifying AZA from neem seeds (Yamasaki et al., 1986), characterizing the insecticidal activities and modes of action of AZA (Barnby and Klocke, 1987), studying the structure-bioactivity relationships of AZA (Yamasaki and Klocke, 1987), and characterizing the stability of AZA and various AZA derivatives (Barnby et al., 1989). More recently, the company has shifted its attention to the formulation of commercial insecticides based on whole neem extracts that contain a spectrum of potentially active ingredients.

Neem extracts contain several compounds that are bioactive against insects. Their combined activities include antifeedant properties, insect growth regulator (IGR) activities, oviposition deterrence effects, and effects on fecundity and overall vigor (Schmutterer, 1990). The first two properties appear to be of greatest commercial interest, and throughout our development program, we have used a variety of laboratory protocols to characterize azadirachtin, AZA derivatives and neem extracts for their antifeedant and IGR effects. These studies include two-way choice leaf disk bioassays, whole plant antifeedant studies, and artificial diet assays. The purpose of this paper is to summarize some of the efficacy data generated to date in our program, and to comment on how characteristics of that efficacy affect the positioning of neem-based products in the insecticide market.

Wood is a Senior Scientist at NPI, 417 Wakara Way, Salt Lake City, Utah, 84108.



## ANTIFEEDANT ACTIVITIES

Neem seed extracts and two of their primary constituents, AZA and salannin, have been shown to deter the feeding of a number of agricultural insect pests (Schmutterer, 1990). We used two-way choice leaf disk bioassays to characterize the antifeedant properties of AZA against fall armyworm (*Spodoptera frugiperda* J.E. Smith), tobacco budworm (*Heliothis virescens* F.) and Colorado potato beetle (*Leptinotarsus decemlineata* Say). In these studies, leaf disks of known area were treated with solutions containing various concentrations of AZA in acetone. After drying, three treated leaf disks and three control leaf disks (acetone only) were placed on moist filter paper in a petri dish. Larvae of the test insect were added to the dish and allowed to feed on the disks for 24 or 48 hours, after which time the amounts of treated and untreated leaf material remaining in each dish were measured. Percent feeding reduction was calculated as:

$$[(\text{percent of control disks eaten} - \text{percent of treated disks eaten}) / \text{percent of control disks eaten}] \times 100.$$

The results of interest in these studies were the concentrations of azadirachtin necessary to reduce feeding on the treated disks by 70%. Those concentrations were 1 mg/l for third instar fall armyworm larvae on lima bean leaf disks, 100 mg/l for fourth instar tobacco budworm larvae on cotton, and 250 mg/l for second instar Colorado potato beetle larvae on potato. Obviously, the effective concentrations necessary to significantly deter feeding varied markedly between insect pests, and very high levels were required for efficacy against some species.

The antifeedant activity of azadirachtin was further characterized using no-choice, whole plant bioassays conducted in the laboratory. In one set of tests, whorl-stage corn plants were sprayed with solutions containing various concentrations of azadirachtin (0-100 mg/l). After the plants had dried they were placed in small cages and infested with third instar fall armyworm larvae. Untreated control plants were handled similarly. Percent feeding reduction and larval mortality were recorded at 3, 5 and 7 days following treatment. Results (Table 1) showed significant and prolonged antifeedant activity against this sensitive pest at concentrations of 20 mg AZA/l and above. No mortality was observed in this seven-day study.

Table 1. Activity of azadirachtin in a long-term, no-choice, whole plant antifeedant test with fall armyworm (*Spodoptera frugiperda*) on corn.

Rate (mg/l)	% Feeding reduction			% Mortality 7 days
	3 days	5 days	7 days	
100	100	98	98	0
50	90	94	87	0
20	83	85	57	0
10	67	65	23	0
0	0	0	0	0

Comparable results were obtained in systemic antifeedant activity assays with fall armyworm on corn. Here, the roots of three week old corn plants were treated with solutions containing AZA at 0-50 mg/l. The plants were again placed in small cages in the laboratory and were infested with third instar larvae. Percent feeding reduction and mortality were assessed at 48 hours. Results (Table 2) again show significant antifeedant activity against this sensitive pest at concentrations of 20 mg AZA/l and above.

Table 2. Systemic antifeedant activity of azadirachtin in a no-choice, whole plant root uptake test with fall armyworm (*Spodoptera frugiperda*) on corn at 48 hours.

Azadirachtin (mg/l)	% Feeding Reduction	% Mortality
50	98	0
20	95	0
10	50	0
1	40	0

While the above laboratory studies indicate that azadirachtin can deter feeding by fall armyworm on corn, related experiments indicate that such activities may be short-lived in the field. In one study, whorl-stage corn plants were sprayed in the field with solutions containing up to 600 mg AZA/l. Leaf materials were collected from these plants at 24 hr and 72 hr post treatment and were bioassayed in the laboratory for feeding deterrence against fall armyworm larvae. Results showed that these samples contained azadirachtin residues in sufficient quantities to deter larval feeding for 24 hours post treatment. After 72 hours in the field, however, azadirachtin residues no longer provided significant feeding deterrence, even on leaves treated with 600 mg AZA/l. The fate of the applied azadirachtin remains unknown. Wash off with rainfall and photodegradation are likely to have contributed to the decline in antifeedant activity (Barnby et al, 1989).

#### INSECT GROWTH REGULATOR ACTIVITY

The ability of neem extracts, and in particular azadirachtin, to interrupt insect growth and development holds considerable commercial potential. Interference with the molting process leads to developmental abnormalities and/or mortality depending on the insect pest under study, the concentrations applied, and the time frame over which observations are made. A broad range of insects within the orders Lepidoptera, Coleoptera, Diptera, Homoptera, and Heteroptera are affected. Lepidopterans appear to be most sensitive (Schmutterer, 1990).

At NPI, we have studied the IGR activities of neem extracts, azadirachtin and azadirachtin derivatives using artificial diet assays. In these tests, a synthetic diet composed of agar, solids, vitamins, water and antibiotics (Kubo and Klocke, 1983) was amended with active extracts or compounds at select concentrations. The diet was then dispensed into small vials along with a single tobacco budworm (Heliothis virescens) larva or fall armyworm (Spodoptera frugiperda) larva. The larvae were allowed to feed, grow and develop for a desired time, after which data concerning mortality and the developmental stages and fresh weights of live larvae were collected. Ten-to-forty replicates were run per treatment. The assay was particularly useful when the IGR activities of numerous compounds and extracts had to be compared. Yamasaki and Klocke (1987) used this artificial diet assay to compare the IGR activities of azadirachtin and two azadirachtin derivatives, 22,23-dihydroazadirachtin and 2',3',22,23-tetrahydroazadirachtin, against first instar larvae of tobacco budworm. The two hydrogenated derivatives are of interest because they show enhanced stabilities against UV degradation and chemical addition (Barnby et al., 1989). Results indicated that, in the absence of prolonged exposure to UV radiation, the two derivatives had the same insecticidal activities as the natural azadirachtin molecule.

Subsequent studies with hydrogenated neem seed extracts containing tetrahydroazadirachtin as the primary active ingredient, support the results of Yamasaki and Klocke (1987). Hydrogenated extracts showed a high degree of potency against third instar fall armyworm larvae (Figure 1) and against first instar tobacco budworm larvae (Figure 2). In both cases, 0.5-1.0 ppm of hydrogenated neem seed extract (PE) in the artificial diet caused significant declines in larval growth and significant increases in larval mortality after five days. Furthermore, these levels of activity were comparable to those measured for the non-hydrogenated extracts (Figure 3), again in the absence of prolonged exposure to UV radiation.

Hydrogenated extracts are expected to have longer residual activities in the field when sprays applied to foliage are exposed to sunlight for extended periods. Barnby et al. (1989) exposed azadirachtin and its dihydro and tetrahydro derivatives to UV light for up to 400 hr and then analyzed the compounds for structural degradation and loss of biological activity. Tetrahydroazadirachtin showed superior structural stability, and the compounds retained biological activity through time in the order tetrahydroazadirachtin > dihydroazadirachtin > azadirachtin.

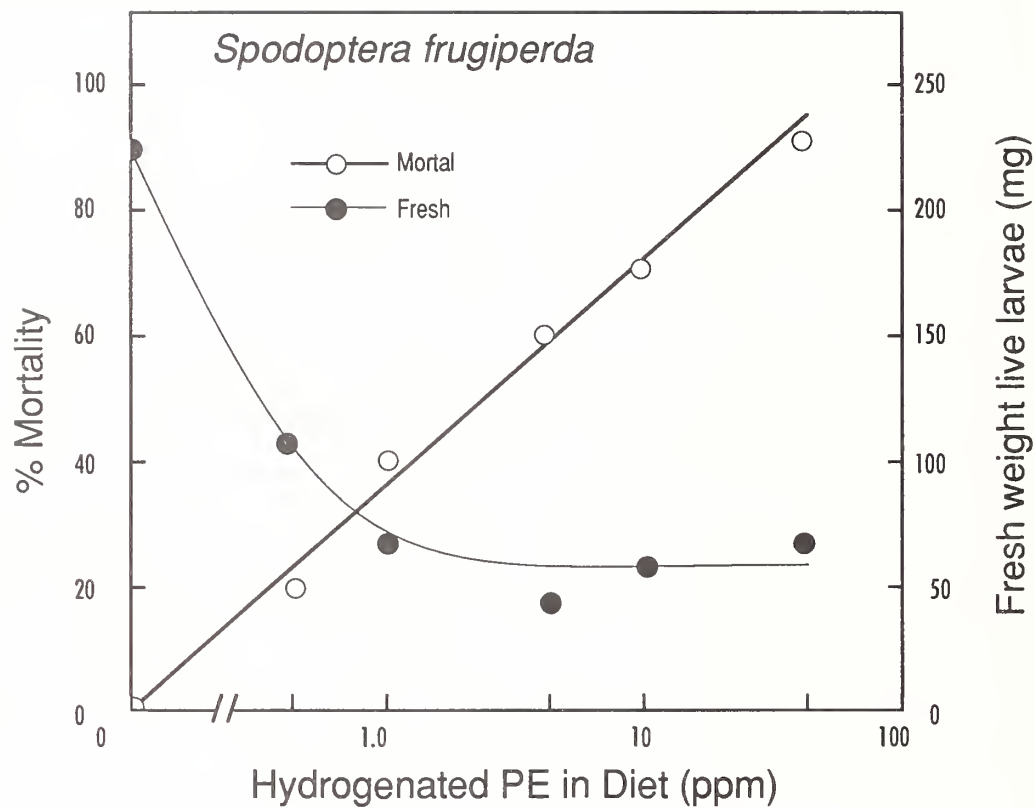


Figure 1. Mortality and fresh weights of *Spodoptera frugiperda* larvae maintained for five days on an artificial diet containing several concentrations of a hydrogenated neem seed kernel extract (PE). The test was initiated with third instar larvae.

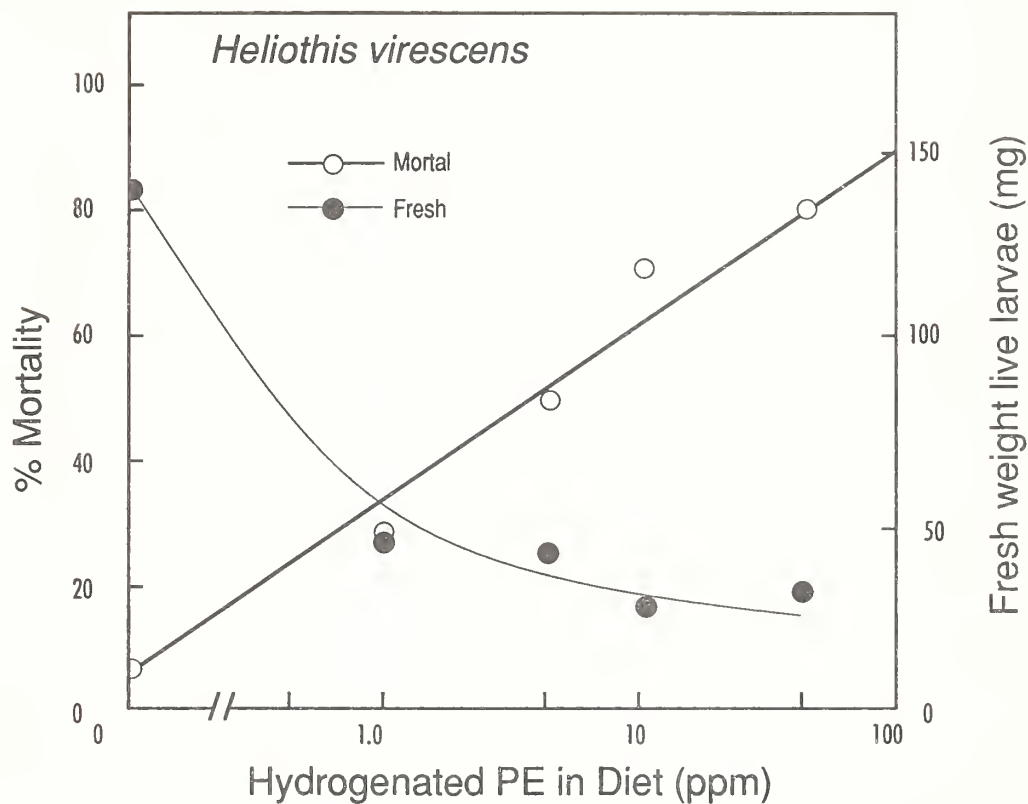


Figure 2. Mortality and fresh weights of *Heliothis virescens* larvae maintained for five days on an artificial diet containing several concentrations of a hydrogenated neem seed kernel extract (PE). The test was initiated with first instar larvae.



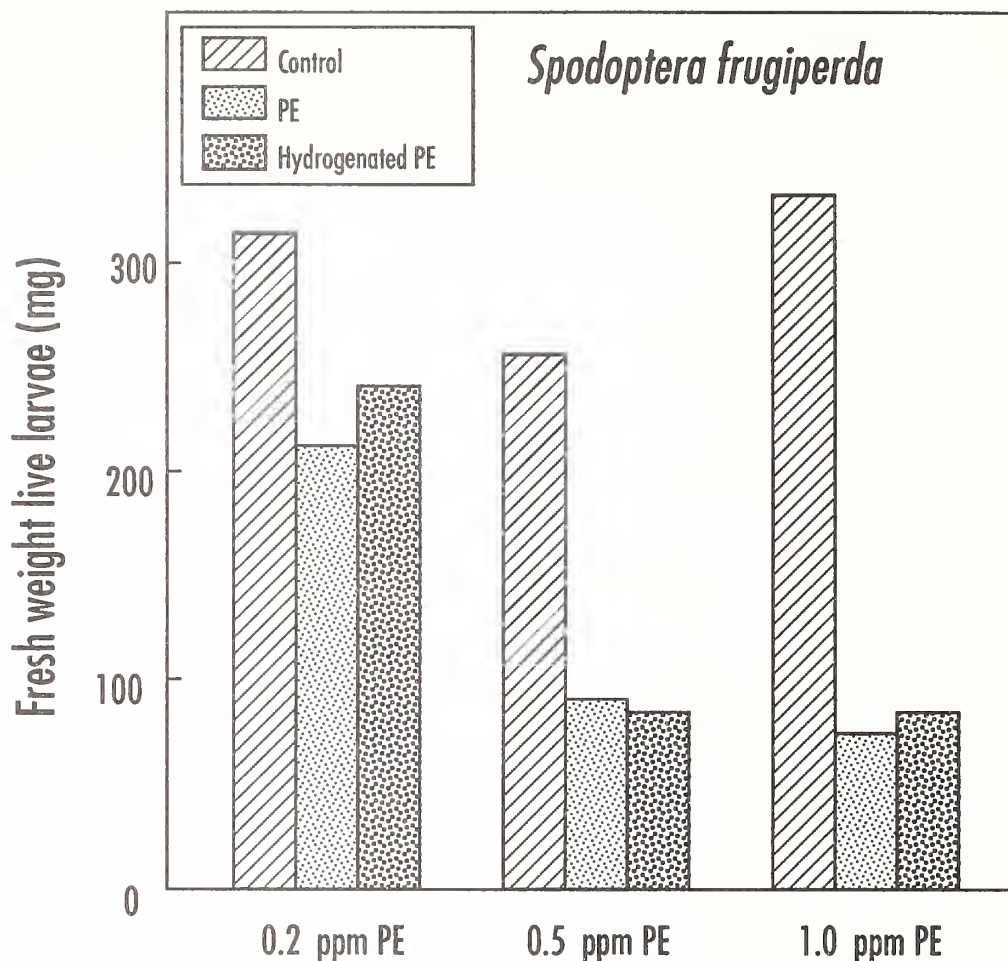


Figure 3. Fresh weight of *Spodoptera frugiperda* larvae maintained for five days on artificial diets containing 0.2, 0.5 and 1.0 ppm of a neem seed kernel extract (PE). No significant differences in IGR activity were seen between hydrogenated and non-hydrogenated extracts.

#### COMMERCIAL CONSIDERATIONS

Efficacy, as a characteristic of insecticides, is a multi-dimensional trait. Components include the numbers of insect taxa that are affected, the insect life stages that are affected, the degree of control that is achieved, the speed with which that control is realized, and the rates and frequencies at which applications must be made. All are important considerations in defining how well an insecticide works.

The laboratory data reviewed here are insufficient to draw detailed conclusions concerning the commercial efficacy of neem-based insecticides. However, these data do address components of that efficacy, and in combination with results reported in the literature, they raise important issues for further discussion and research. Three such issues are addressed below.

First, the antifeedant activity of neem extracts is an interesting property, but its commercial significance may be limited. There appears to be considerable variation between insect pests in sensitivity to azadirachtin as an antifeedant, and high AZA concentrations are required to deter feeding by many species. Furthermore, there is some evidence that antifeedant activities may be short lived under field conditions. This constraint can

potentially be reduced by improving the stability of azadirachtin through hydrogenation. Nevertheless, commercial use of neem-based products as antifeedants may be limited to narrow market segments. Second, IGR activities appear to be the most important commercial characteristics of neem-based insecticides. Many economically important insect pests are affected, and fairly high levels of control are realized at relatively low application rates. Importantly, this mode of action does not afford quick kill. Effects are typically seen 3-15 days following treatment depending on the target insect and the timing of application. Furthermore, IGR activity is limited to stages of development when the insect is actively molting. Typically there is no adult or egg mortality. In many respects, azadirachtin should be considered as a larvicide.

These properties do not impose serious constraints on product efficacy, but they may pose challenges for commercialization in markets where quick adult knockdown is important or expected. Such challenges can be met in part through appropriate positioning of the product and through grower education. But, as Isman et al. (1990) point out, there will be segments of the insecticide market where neem-based products will not provide adequate control.

Third, the systemic activity of azadirachtin deserves additional study. It is clear that many plants can take up AZA from soil and transport the compound to their shoots where bioactivity is expressed. Questions remain, however, as to whether systemic activity is widespread throughout the range of important agricultural and horticultural crops, whether there is much uptake through foliage, whether translaminar movement from upper to lower leaf surfaces is common, and whether azadirachtin is phloem mobile. Widespread and quantitative systemic activity would represent an important component of product efficacy and could be used to enhance the versatility of neem-based insecticides.

Public concerns with agricultural pollution have placed a premium on development of environmentally-sound pest management strategies. From the standpoint of safety, neem-based insecticides, with their low mammalian toxicities, offer attractive alternatives to many hard chemical pesticides in use today. However, if they are to enjoy widespread use, neem-based products must demonstrate efficacy that is competitive with existing chemicals. Neem insecticides hold the potential to do just that. The challenges that lie ahead are in formulation and product positioning. Neem-based products must be formulated for maximum biological efficacy and stability, and they must be targeted toward markets where their efficacy is competitive.

#### LITERATURE CITED

- Barnby, M.A. and J.A. Klocke. 1987. Effects of azadirachtin on the nutrition and development of the tobacco budworm, Heliothis virescens (Fabr.) (Noctuidae). J. Insect Physiol., 33:69-75.
- Barnby, M.A., R.B. Yamasaki and J.A. Klocke. 1989. Biological activity of azadirachtin, three derivatives, and their ultraviolet radiation degradation products against tobacco budworm (Lepidoptera:Noctuidae) larvae. J. Econ. Entomol., 82:58-63.
- Isman, M.B., O. Koul, D.T. Lowery, J.T. Arnason, D. Gagnon, J.G. Stewart and G.S. Salloum. 1990. Development of a neem-based insecticide in Canada. (This volume).
- Schmutterer, H. 1990. Properties and potential of natural pesticides from the neem tree, Azadirachta indica. Annu. Rev. Entomol., 35:271-297.
- Yamasaki, R.B. and J.A. Klocke. 1987. Structure-bioactivity relationships of azadirachtin, a potential insect control agent. J. Agric. Food Chem., 35:467-471.
- Yamasaki, R.B., J.A. Klocke, S.M. Lee, G.A. Stone and M.V. Darlington. 1986. Isolation and purification of azadirachtin from neem (Azadirachta indica) seeds using flash chromatography and high-performance liquid chromatography. J. Chromatog., 356:220-226.

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RESPONSES OF SWEETPOTATO WHITEFLY TO AZADIRACHTIN  
EXTRACTED FROM NEEM TREE SEEDS (AZADIRACHTA INDICA)

James F. Price and David J. Schuster

ABSTRACT

Egg, second to early third stage nymph, late third to early fourth stage nymph, late fourth stage nymph ("pupa") and adult sweetpotato whiteflies (Bemisia tabaci (Gennadius)) developing on poinsettia leaves were treated with Margosan-O preparations of azadirachtin extracted from neem tree seeds. Single foliar spray applications of 20 ppm azadirachtin to the above life stages resulted in 4.0%, 96.0%, 74.0%, 40.7% and 8.0% mortality respectively. Nymphs hatching from eggs treated with the spray were not killed. In another experiment, 20 or 40 ppm preparations were applied to second and third stage nymphs one to three times at 3 day intervals. Mortality of immature sweetpotato whiteflies was higher at 40 ppm than at 20 ppm. At 20 ppm, mortality was increased by a second application but mortality was not increased by a second application at 40 ppm. Two-hundred and thirty-seven milliliters of 20 ppm azadirachtin applied once as a soil drench to 15 cm diameter pots of poinsettias infested with second stage nymphs did not increase mortality significantly among the insects. Four weekly applications of Margosan-O at 28 ppm azadirachtin did not damage gerbera daisy, Persian violet, gloxinia nor African violet. Four weekly applications of Margosan-O at 38 ppm azadirachtin did not damage any of five poinsettia cultivars. Commercial azadirachtin can be a useful tool to manage sweetpotato whitefly on ornamental crops.

INTRODUCTION

The azadirachtin extracted from seeds of the neem tree (Azadirachta indica A. Juss.) has been available for development as an insecticide for several years and properties of neem seed extracts to affect the behavior and development of insects recently have been summarized by Schmutterer (1990). Effects of these extracts upon arthropods injurious to ornamentals have been reported by Knodel et al. (1986), Larew et al. (1985) Webb et al. (1983), and others. Coudriet et al. (1985) found that applications of 2% aqueous solutions of neem seed extract to sweetpotato whitefly (Bemisia tabaci (Gennadius)) resulted in reduced egg viability and oviposition, prolonged larval periods and larval mortality. They believed that the extracts acted as an antiecdysteroid or may have affected the neuroendocrine control of ecdysteroids. Flint and Parks (1989) found that 160 ppm azadirachtin applied in aqueous sprays to sweetpotato whitefly on cotton resulted in 60% reductions in numbers of immatures, but at 20 ppm sprays were ineffective.

A commercial preparation of azadirachtin, Margosan-O (Grace-Sierra, Fogelsville, PA.), is within the process for registration for use on ornamental crops. This paper reports research conducted in 1988 and 1989 to determine the usefulness of the commercial preparation of azadirachtin for management of sweetpotato whitefly on poinsettia and other ornamental crops.

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Price is Associate Professor (Entomology) and Schuster is Professor (Entomology), University of Florida, IFAS, Gulf Coast Research and Education Center, Bradenton, FL.



## MATERIALS AND METHODS General

Insects used in these experiments were sweetpotato whiteflies from a laboratory colony held for about 2 years on poinsettia. The original stock was from poinsettia naturally infested in Bradenton, FL. Plants used in product efficacy experiments were about six weeks old 'V-14 Glory' poinsettias grown from rooted cuttings supplied by Paul Ecke Poinsettia Ranch, Encinitas, California. Poinsettias were grown one single stem plant in each 15 cm pot.

### Experiment 1.

A randomized complete block experiment in four replications was designed to detect effects of 20 ppm azadirachtin to various lifestages of the sweetpotato whitefly. Poinsettias were reduced to two middle leaves and were placed into an insectary where more than 5,000 adult sweetpotato whiteflies were released. Adult females were allowed to lay eggs on undersides of leaves for 1 day after which all adults were collected with a vacuum pump aspirator. As the insects developed to the second and early third nymphal stage ("small nymphal"), late third and early fourth nymphal stage ("large nymphal"), or late fourth nymphal ("pupal") stage, twenty-five insects per leaf (one experimental unit) were encircled with a 5 mm diameter circle made from a laboratory marking pen and experimental treatments were applied. Experimental treatments were an untreated check, bifenthrin (Talstar 10WP) 120 ppm bifenthrin (a standard for use in the Florida poinsettia industry), or 20 ppm azadirachtin. Treated plants were returned to the greenhouse and the insects were allowed to develop to the adult stage or die. After insects had died or emerged as adults, numbers of dead immatures or exuviae remaining after emergence of adults were counted to provide percentage mortality.

Pesticide effects on adults were evaluated by treating the upper and lower surfaces of leaves of poinsettia plants, allowing the leaves to dry in the greenhouse for 1 hour, then removing four middle leaves. Petioles were placed in a water vial and leaves and vials were placed into a 1.43 liter, clear plastic container outfitted with a ventilating screen top and side. Twenty five adult sweetpotato whiteflies were introduced onto the leaves. Numbers of live and dead adults were counted 48 hours later.

Data were transformed by the arcsin transformation and analyzed by an analysis of variance. Means were calculated and means separations were provided by Duncan's New Multiple Range Test.

### Experiment 2

A 2X3 factorial experiment was designed in four replicates to detect effects of 20 ppm and 40 ppm azadirachtin applied once, twice or three times to sweetpotato whitefly nymphs. A group of plants was prepared to have whitefly eggs as in Experiment 1. As the insects reached the early second nymphal stage, first applications of experimental treatments were applied. Three days later, plants to receive two or three applications were treated a second time and after an additional 3 days, plants to receive three applications were treated. Post treatment evaluations and data analysis were performed as in experiment 1.



### Experiment 3

A randomized complete block experiment was designed in four replications to detect systemic effects of soil applied azadirachtin and other systemic insecticides on nymphal sweetpotato whiteflies. A group of plants was prepared to have whitefly eggs as in experiment 1 except that no leaves were removed from the plants. When the insects reached the early second stage, insecticidal treatments were applied to the soil. Insecticidal treatments were 237 ml of 20 ppm azadirachtin, 237 ml of a 304 ppm preparation of oxamyl (Vydate L), 0.16 g of formulated oxamyl (Oxamyl 10G), 0.15 g of formulated aldicarb (Temik 10G), and 0.11 g of formulated disulfoton (Disyston 15G) applied to the soil of each pot and watered. Post treatment evaluations of the experiment and data analysis were performed as was done in Experiment 1 except that mortality response was determined from 25 encircled immatures on each of an upper, middle, and lower leaf.

### Experiment 4

A randomized complete block experiment in four replications was designed to evaluate the phytotoxic reaction of selected greenhouse ornamental plants to azadirachtin sprays. Preparations of 28 ppm azadirachtin were applied as sprays to groups of 24 'Small Inc. Mix' gerbera daisies, 24 'Blue Champion' and 6 'White Champion' Persian violets, 24 'Super Compact Mix' gloxinias, and 24 'Small Inc. Mix' African violets. Similar untreated checks were provided. Treatments were applied weekly for 4 weeks beginning 6 June 1989 and an additional treatment was applied to open flowers as they appeared. Applications were made with a hand-held spray gun delivering ca. 14 kg/sq. cm pressure and 1870 liters/ha. After each spray application, the conditions of treated plants were compared to those of the untreated check.

### Experiment 5

A randomized complete block experiment in four replications was designed to evaluate the phytotoxic reaction of selected cultivars of shadehouse grown poinsettias. Preparations of 38 ppm azadirachtin were applied to groups poinsettias that included 3 of each of 38 cm tall, multistem 'Annette Hegg Lady', Gutbier 'V-14 Glory', Gutbier 'V-10 Amy', Gutbier 'V-17 Angelika', and Gross 'SUPJIBI' poinsettias. Untreated checks were provided. Treatments were applied weekly for 4 weeks beginning 12 June 1989, before plants were showing color. Applications were made using a hand pumped knapsack sprayer that delivered about 2800 liters/ha. After each spray, the conditions of treated plants were compared to those of the untreated checks.

## RESULTS

### Experiment 1

Results of Experiment 1 are presented in Table 1. High levels of mortality occurred among small nymphs and among large nymphs treated with azadirachtin, with the highest level occurring when small nymphs were treated. A moderate level of mortality occurred when pupae were treated with azadirachtin. Compared to the untreated check, there was no significant increase in mortality to sweetpotato whitefly eggs, or adults treated with 20 ppm azadirachtin, nor to nymphs hatching from eggs previously treated with 20 ppm azadirachtin. Efficacious 20 ppm azadirachtin is inconsistent with the results found by Flint and Parks (1989). Perhaps the greenhouse experiment reported herein provided superior nymphal contact with the toxicant. Bifenthrin treatments resulted in increased mortality, compared to the untreated checks, when applied to small nymphs and adults. Bifenthrin also resulted in increased mortality to nymphs that emerged from eggs that had been treated.

### Experiment 2

Results of Experiment 2 are presented in Table 2. There was a significant interaction between concentration of azadirachtin in the spray preparation and the number of times the preparations were applied. Mortality of the immature whiteflies was higher at 40 ppm azadirachtin than at 20 ppm and there was no additional mortality at 40 ppm when the preparation was applied more than once. At the 20 ppm concentration, mortality of immature whiteflies increased when a second application was performed; a third application did not increase mortality further. All treatment combinations resulted in increased mortality compared to the untreated check.

### Experiment 3

Results of Experiment 3 are presented in Table 3. There was an unusually high mortality in the untreated check (37%), the reasons for which can not be explained satisfactorily. Only two treatments resulted in higher mortality than was found in the untreated checks, aldicarb and oxamyl L. There was no significant increase in mortality, compared to the untreated checks, when azadirachtin, disulfoton or oxamyl 10G were applied.

### Experiments 4 and 5

No phytotoxic damage was found on any of the foliage or flowers of plants to which azadirachtin was applied.

The results of Experiments 1-5 indicate that azadirachtin applied as a spray at 20-40 ppm can be useful to control the nymphal stage sweetpotato whitefly. Since most greenhouse populations of the insect contain a complex of all lifestages, additional applications of azadirachtin or concomitant applications of an effective adulticide would be required to achieve control. The plant safety on a variety of poinsettias and other flower crops indicates that large segments of the ornamentals industry likely could use the commercial azadirachtin. Azadirachtin can become an important tool for the management of sweetpotato whitefly, certainly on poinsettia and perhaps on other ornamentals as well.

## REFERENCES

- Coudriet, D. L., N. Prabhaker, and D. E. Meyerdirk. 1985. Sweetpotato whitefly (Homoptera:Aleyrodidae): Effects of neem-seed extract on oviposition and immature stages. *Environ. Entomol.* 14(6):776-9.
- Flint, Hollis M. and Nancy J. Parks. 1989. Effect of Azadirachtin from the neem tree on immature sweetpotato whitefly, Bemisia tabaci, (Homoptera:Aleyrodidae) and other selected pest species on cotton. *J. Agric. Entomol.* 6(4):211-5.
- Knodel, Janet J., Hiram G. Larew, and Ralph E. Webb. 1986. Margosan-O, a commercial formulation of neem seed extract, controls Liriomyza trifolii on chrysanthemums. *J. Agric. Entomol.* 3(3):249-54.
- Larew, Hiram G., J. J. Knodel-Montz, Ralph E. Webb, and J. David Warthen. 1985. Liriomyza trifolii (Burgess) (Diptera:Agromyzidae) control on chrysanthemum by neem seed extract applied to soil. *J. Econ. Entomol.* 78(1):80-4.
- Schmutterer, H. 1990. Properties and potential of natural pesticides from the neem tree, Azadirachta indica. *Ann. Rev. Entomol.* 35:271-97.
- Webb, Ralph E., Mark A. Hinebaugh, Richard K. Lindquist, and Martin Jacobson. 1983. Evaluation of aqueous solutions of neem seed extract against Liriomyza sativae and L. trifolii (Diptera:Agromyzidae). *J. Econ. Entomol.* 76(2):357-62.

Table 1. Percent of sweetpotato whitefly lifestages on poinsettia that died when the indicated lifestage was treated with aqueous sprays of 20 ppm azadirachtin or 120 ppm bifenthrin or was left untreated. First instars were not treated but emerged from eggs that had been treated.

Treatment	Egg	First Instars	Small Nymphs	Large Nymphs	Pupae	Adults
Untreated	0 A	0 B	6 B	8 B	0 B	4 B
Bifenthrin	0 A	87 A	98 A	12 B	1 B	95 A
Azadirachtin	0 A	4 B	96 A	74 A	41 A	8 B

Values within a column and followed by the same letter are not significantly different (5% level) by Duncan's New Multiple Range Test.

Table 2. Interactive effects of aqueous sprays of 20 ppm or 40 ppm azadirachtin applied to nymphal sweetpotato whiteflies on poinsettia one, two or three times.

Treatment		No. Times Applied	% Dead Immatures
Untreated		-----	7 D
Azadirachtin	20 ppm	Once	27 C
Azadirachtin	20 ppm	Twice	55 B
Azadirachtin	20 ppm	Thrice	55 B
Azadirachtin	40 ppm	Once	83 A
Azadirachtin	40 ppm	Twice	91 A
Azadirachtin	40 ppm	Thrice	88 A

Values followed by the same letter are not significantly different (5% level) by Duncan's New Multiple Range Test.

Table 3. Percent of immature sweetpotato whiteflies on poinsettia grown in 15 cm diameter pots that died after soil treatments with the indicated systemic insecticides.

Treatment	% Dead Immatures
Untreated	37 B
Azadirachtin, 237 ml of a 20 ppm ai preparation	41 B
Aldicarb (Temik) 10G, 0.15 g formulation	72 A
Oxamyl (Vydate L), 237 ml of 304 ppm ai preparation	72 A
Oxamyl (Oxamyl 10G), 0.16 g formulation	42 B
Disulfoton (Disyston) 15G, 0.11 g formulation	40 B

Values followed by the same letter are not significantly different (5% level) by Duncan's New Multiple Range Test.

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LABORATORY AND GREENHOUSE EVALUATIONS OF MARGOSAN-O AGAINST  
BIFENTHRIN-RESISTANT AND -SUSCEPTIBLE GREENHOUSE WHITEFLIES,  
TRIALEURODES VAPORARIORUM (HOMOPTERA: ALEYRODIDAE)

R.K. Lindquist, A.J. Adams, F.R. Hall and I.H.H. Adams

ABSTRACT

Three types of bioassays were used to evaluate Margosan-O's activity against greenhouse whitefly, Trialeurodes vaporariorum, (GHWF) larvae on poinsettia plants: high-volume "wet" sprays to whole plants; adaxial and abaxial leaf surface treatments with cotton swabs or hand-sprayers; and a uniform drop generator applying different numbers, sizes and concentrations of drops/cm<sup>2</sup>. The high-volume spray experiments utilized only bifenthrin-resistant insects, whereas both resistant and susceptible GHWF were included in other assays. There were no differences in larval mortality or adult emergence between the two GHWF populations. Applying Margosan-O only to adaxial leaf surfaces had little effect on whitefly mortality or emergence, except at very high concentrations. Based on results using a uniform spray drop generator, the effects of Margosan-O on GHWF larvae were dose-dependent, rather than based on numbers of drops/cm<sup>2</sup>. Margosan-O delayed adult GHWF emergence for several days, compared with untreated insects.

INTRODUCTION

Extracts of seeds produced by the neem tree (Meliaceae: Azadirachta indica) have been evaluated as repellents, anti-feedants, insect growth regulators and insecticides against a wide range of insect pests (Jacobson, 1986; Schmutterer, 1985). Activity has been demonstrated against numerous agricultural pests such as the Egyptian cotton leafworm, Spodoptera littoralis, (Meisner et al., 1983), Colorado potato beetle (Zehnder and Warthen, 1988), Mediterranean fruit fly (Steffens and Schmutterer, 1982), leafminer, Liriomyza trifolii (Larew et al. 1985; Lindquist et al. 1986) and sweetpotato whitefly, Bemisia tabaci, (Prabhaker et al., 1989). Most of these studies utilized crude extracts from seeds, diluted with acetone, methanol or water.

A commercial formulation of neem seed extract, Margosan-O, containing 3 g/l azadirachtin, has been under development for several years, and will soon be marketed in the USA by Grace-Sierra Company. Margosan-O has shown good efficacy against L. trifolii larvae, applied either as a foliar spray or growing medium drench (Lindquist et al., 1988; Knodel et al., 1986). We have been evaluating Margosan-O for the past two years against several other important arthropod pests that affect greenhouse crops, including the greenhouse whitefly, Trialeurodes vaporariorum (GHWF). We report here results of greenhouse and laboratory experiments to evaluate Margosan-O against GHWF larvae on poinsettia (Euphorbia pulcherrima). Both pyrethroid-resistant (bifenthrin) and susceptible populations were included in several experiments.

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Lindquist and Hall are professors, Laboratory for Pest Control Application Technology, Department of Entomology, The Ohio State University and Ohio Agricultural Research and Development Center (Wooster, Ohio) 44691; A. Adams was a visiting research scientist and I. Adams was a research assistant at the above location. A. Adams is presently with Wellcome Environmental Health, Berkhamsted, England.



## MATERIALS AND METHODS

Two GHWF populations were used. One was collected from greenhouse ornamental plants maintained at the Ohio Agricultural Research and Development Center (OARDC). The population had been exposed to numerous insecticides, especially pyrethroids, but its resistance characteristics were not known until a series of experiments with bifenthrin (Capture 2EC, FMC Corp., Princeton, NJ; Adams et al., unpublished data) showed that much higher than recommended rates were necessary to obtain adequate control. The second population was pyrethroid-susceptible, obtained from Dr. John Sanderson, Department of Entomology, Cornell University. Bifenthrin was 100X more effective against this population, compared with the OARDC population. Both populations were maintained on tobacco plants without any insecticide pressure in separate greenhouses.

High-volume sprays. Preliminary experiments, using high-volume (HV) sprays were conducted with potted poinsettia plants, cv 'Brilliant Diamond' (supplied by Paul Ecke Poinsettias, Encinitas, CA), grown in 10 cm diam. plastic pots. These experiments were conducted to obtain estimates of application rates and intervals required to control GHWF on poinsettia and utilized only resistant whiteflies. Poinsettias were exposed to adult GHWF in 80 x 60 x 45 cm screened cages for 72h to obtain eggs. Adults were then removed by shaking plants and treating any remaining adults with a resmethrin aerosol application in a spray chamber. Plants were then placed in cheesecloth holding cages to await egg hatch. Sprays were generally applied to settled first-instar larvae, but later instars were also treated in some experiments. The number of larvae present on two leaves/plant, (those containing the highest number of larvae) was counted prior to treatment. Two or three applications were made, using a CO<sub>2</sub>-powered sprayer at 207 kpa and a hollow-cone nozzle. Both abaxial and adaxial leaf surfaces were sprayed to run-off, with the total spray volume ca. 2700 liters/ha. Any subsequent applications were made at 7-day intervals. There were four or five single-plant replicates of each treatment. After applications, plants were removed to a cheesecloth cage to await larval mortality and adult emergence counts.

When the majority of GHWF larvae on untreated plants were in the last instar, marked leaves were removed from all plants and larval mortality was recorded using a binocular microscope. Because Margosan-O may act as an insect growth regulator and GHWF larvae are sessile, a larva was considered dead if it had not molted. Leaves were then placed on moist subirrigation matting in trays, and adult emergence was subsequently recorded, generally up to 25-28 days post-treatment. Per cent mortality and adult emergence were then calculated, based on pre-treatment counts. Data were subjected to analysis of variance.

Applications to Abaxial and Adaxial Leaf Surfaces. Two groups of experiments were conducted, one group with "wet" applications, using swabs and hand sprayers, and another using a uniform spray drop generator. Both resistant and susceptible populations were included. In the "wet" applications, poinsettia plants, cv. 'Hot Pink', were infested by exposing them to adult GHWF for 24h. After infestation and adult GHWF removal, plants were placed under fluorescent lights (LD, 16:8) at 25-30°C until settled first-instar larvae were present (7 to 10 days). After making pre-treatment counts, Margosan-O was applied to either the abaxial surface using a small hand pump sprayer, or to the adaxial surface with cotton swabs. Two concentrations, 2 and 6.25 mls/liter (0.006 and 0.019 g a.i./l, respectively), were used.

Leaf surfaces were thoroughly wetted with each application. Ten adaxial/abaxial leaf surfaces containing larvae from each GHWF population were treated with the 2 mls/liter concentration, and four were treated with the 6.25 mls/liter concentration. Plants were held in the laboratory on a light bench at 24-28°C. Larval mortality and adult emergence were recorded at intervals after treatment. After 21 days a final figure for adult emergence was obtained. Data were then subjected to analysis of variance.

**Uniform drop generator.** In order to better estimate drop the numbers, sizes and concentrations required to obtain a certain per cent mortality, several experiments were conducted using an on-demand drop generator (Reynolds et al., 1987). The Margosan concentrations selected were among those used in several experiments with high- and low-volume application equipment we were conducting (to be published elsewhere). Poinsettia plants, cv. 'Brilliant Diamond', were infested with either resistant or susceptible GHWF, and handled as described previously. A 5cm<sup>2</sup> tube dipped in ink was used to mark circles on abaxial leaf surfaces, and all larvae within the circles were counted prior to treatment. The drop generator was used to apply a pre-determined number of uniform-sized drops containing different Margosan-O concentrations within these circles. A wetting agent (Ortho X-77, 0.1% v/v) was added to solutions.

In the first experiments Margosan-O concentrations of 2, 20 and 50 mls/liter (0.006, 0.06 and 0.15 g a.i./l), in 100um drops, at densities of 25, 50, 100, and 200 drops/cm<sup>2</sup> were applied to abaxial, and adaxial leaf surfaces. Each combination of drop density, leaf surface and Margosan concentration was replicated four times. Adult emergence was assessed at 16 days post-treatment.

In another experiment Margosan-O was applied as above, but only to abaxial leaf surfaces, at 35 mls/liter, using three drop sizes (70, 100, and 150um) and drop densities of 12, 25, 50, 100, and 150/cm<sup>2</sup>. GHWF mortality and adult emergence were assessed 7 and 21 days post-treatment. GHWF mortality after 7 days, in the above experiment, using the 150um drop data and logit analysis, was used to calculate an LN<sub>50</sub> of 53 drops/cm<sup>2</sup>. This was used to estimate a "standard" Margosan dose/unit area of 3.3 nl(0.0099ng/cm<sup>2</sup>) margosan/cm<sup>2</sup>. The drop generator was then used to apply the same dose/cm<sup>2</sup> by varying drop size, number and concentration. In one application the Margosan concentration remained the same at 35 mls/l, and drop size and drops/cm<sup>2</sup> were varied. In another application, the no. of drops/cm<sup>2</sup> remained constant at 53, and the Margosan-O concentration and drop sizes were varied. These experiments were conducted to ascertain if drop size, concentration or density affected Margosan's efficacy against GHWF larvae, or whether efficacy was strictly dose-dependent.

In a subsequent assay, infested 10 cm<sup>2</sup> leaf discs were sprayed with a solution of water, X-77 (0.1% v/v) and Saturn Yellow (Day-Glo, a fluorescent pigment tracer, 3 g/l) to determine whether the spray was reaching the target. First-instar GHWF larvae were counted on each disc and sprayed using the drop generator with three drop sizes (100, 150 and 250um) and seven drop densities, from 3 to 150/cm<sup>2</sup>. Leaf discs were then examined under ultra-violet illumination and drops and "hit" larvae were counted. The percentage of hits was calculated for each treatment.

# RESULTS AND DISCUSSION

High-volume sprays. Results of the high volume spray applications are presented in Tables 1-3. Three Margosan applications, 7 days apart, at 6.2 and 12.5 mls/l (50.2 and 100.4 gms azidirachtin/ha/application, respectively, at the spray volume of 2700 l/ha used), resulted in complete control of GHWF (Table 1). No adults had emerged on plants treated with either concentration by the last sample date. Two applications, 7 days apart, at 3.1 and 6.2 mls/l (25.1 and 50.2 gms azidirachtin/ha/application) also provided excellent control (Table 2). None of the whiteflies on leaves treated with either Margosan-O rate had emerged by the last sample date, compared with 85.4% on untreated plants. Even a single application at 0.8 mls/l (6.2 gms azidirachtin/ha) significantly reduced adult emergence (Table 3).

Table 1. Control of greenhouse whitefly larvae on Poinsettia with three high-volume spray applications of Margosan-O .

Treatment <sup>1</sup>	Rate <sup>2</sup> (ml/l)	No. Live Larvae <sup>3</sup>		% Emerged Adults <sup>3</sup>
		Pre-treat	Day 18 <sup>4</sup>	Day 23 <sup>4</sup>
Margosan-O	6.2	31.1a	1.0a	0a
Margosan-O	12.5	23.0a	3.2a	0a
Untreated		83.4b	66.8b	85.4b

<sup>1</sup>Three applications, on days 1, 8 and 16; The first application was to settled 1st-instar larvae.

<sup>2</sup>At a spray volume of 2700 liters/ha, the 6.2 ml conc.=50.2 gms azidirachtin/ha.

<sup>3</sup>Means in each column followed by the same letter(s) do not differ significantly (P=0.05) according to Duncan's NMRT.

<sup>4</sup>Day after first application.

Table 2. Control of greenhouse whitefly larvae on poinsettia with two high-volume spray applications of Margosan-O .

Treatment <sup>1</sup>	Rate <sup>2</sup> (ml/l)	No. live larvae <sup>3</sup>		% Emerged Adults <sup>3</sup>
		Pre-treat	Day 14 <sup>4</sup>	Day 22 <sup>4</sup>
Margosan-O	3.1	59.2a	5.2a	1a
Margosan-O	6.2	38.6a	1.2a	0a
Margosan-O	12.5	78.2a	.8a	0a
Untreated		118.6a	116.6b	75.8b

<sup>1</sup>Two applications, on days 1 and 8; The first application was to settled 1st-instar larvae.

<sup>2</sup>At a spray volume of 2700 liters/ha, the 3.1 ml/l concentration =25.1 gms azidirachtin/ha.

<sup>3</sup>Means in each column followed by the same letter(s) do not differ significantly (P=0.05), according to Duncan's NMRT.

<sup>4</sup>Day after first application.

Table 3. Control of greenhouse whitefly larvae on poinsettia with one Margosan-O high-volume spray application.

Treatment <sup>1</sup>	Rate <sup>2</sup> (ml/l)	No. live larvae <sup>3</sup>		% Emerged Adults <sup>3</sup>
		Pre-treat	Day 18 <sup>4</sup>	Day 18 <sup>4</sup>
Margosan-O	1.6	47.0a	5.0a	12.2a
Margosan-O	0.8	39.2a	6.0a	14.2a
Untreated		55.0a	43.5b	77.2b

<sup>1</sup>Application on day 1, to settled 1st-instar larvae.

<sup>2</sup>At a spray volume of 2700 liters/ha, the 1.6 ml/l concentration=12.5 gms azadirachtin/ha.

<sup>3</sup>Means in each column followed by the same letter(s) do not differ significantly (P=0.05) according to Duncan's NMRT.

<sup>4</sup>Day after first application.

These experiments showed that this formulation of Margosan-O was effective in controlling bifenthrin-resistant GHWF on poinsettias, causing both direct larval mortality and reduced adult emergence. According to Grace-Sierra, the recommended rate of azadirachtin is 25 gms/ha (J.F. Knauss, Pers. Comm.). GHWF control was obviously better with repeat applications of this and higher rates, compared with a single application of 0.5 and 0.25X the normal rate. However, even relatively low application rates caused significant effects on GHWF larvae.

**Applications to Abaxial and Adaxial Leaf Surfaces.** Both application rates were quite effective in the "wet" spray experiments, when Margosan was applied to abaxial surfaces. However, mortality was higher and adult emergence was lower at the higher Margosan concentration (Table 4). Applications to adaxial surfaces were ineffective, showing that, at these concentrations, Margosan had no significant translaminar activity against GHWF larvae on poinsettia. Margosan was equally effective against bifenthrin-resistant and susceptible GHWF. Thus, there is no evidence of cross-resistance to Margosan. Both whitefly populations were used interchangeably in subsequent experiments.

Table 4. Greenhouse whitefly mortality and adult emergence after a single Margosan-O application at two concentrations to adaxial and abaxial surfaces of poinsettia leaves containing bifenthrin-resistant or -susceptible first-instar larvae.

GHWF Population	Leaf Surface	% Mortality <sup>1</sup> (Day 7) <sup>2</sup>		% Adult Emergence <sup>1</sup> (Day 21) <sup>2</sup>	
		2ml/l	6.25ml/l	2ml/l	6.25ml/l
Susceptible	Adaxial	14.0a	25.5a	64.7b	41.4ab
	Abaxial	55.5b	91.2b	11.1a	0 a
	Untreated	11.8a	15.5a	75.3b	47.0b
Resistant	Adaxial	14.6a	11.7a	63.9b	60.7b
	Abaxial	58.0b	85.1b	13.5a	0.9a
	Untreated	15.8a	13.2a	70.2b	63.9b

<sup>1</sup>Means in each column for resistant or susceptible whiteflies followed by the same letter(s) do not differ significantly (P=0.05), according to Duncan's NMRT.

<sup>2</sup>Day post-treatment.



Uniform drop generator. Table 5 shows results of a single Margosan application to abaxial leaf surfaces containing first-instar larvae of the resistant population. There was little mortality after 9 days at 2 mls/liter, regardless of drop density (12.8-18.5%). This is in contrast to mortality in the "wet" applications at this concentration (Table 4). At the other concentrations there was higher mortality at higher drop densities (=higher dosage/cm<sup>2</sup>). The per cent adult emergence after 16 days was not related to drop density at the lowest concentration, but was approximately 50% of that on untreated leaves. As with larval mortality, adult emergence was related to concentration and drop density at the two higher Margosan concentrations. In addition to direct mortality, there may be significant sublethal effects associated with Margosan-O treatments against GHWF. GHWF larvae on treated leaves were generally smaller compared with those on untreated leaves, and adults were slower to begin emerging as well, particularly on leaves treated with the higher concentrations. This delay often was two or more days. Some of the adults on treated leaves died on emerging or did not appear normal if they did emerge. This has been reported for sweetpotato whitefly, *B. tabaci* (Prabhaker et al., 1989) as well as other insect species.

Table 5. Greenhouse whitefly larval mortality and adult emergence after a single application of Margosan-O using 100u drops at three concentrations and four densities to abaxial surfaces of poinsettia leaves (Resistant GHWF).

Margosan Conc. (ml/l)	Drop Densities (no/cm <sup>2</sup> )	N	% Mortality	% Adult Emergence
			Day 9	Day 16
2	25	446	14	34
	50	216	16	44
	100	113	13	50
	200	125	19	48
20	25	81	24	36
	50	88	38	27
	100	75	36	14
	200	43	67	2
50	25	167	30	16
	50	236	72	6
	100	169	62	6
	200	152	86	0
untreated		266	0	85

When applications at the same concentrations were made to adaxial surfaces, mortality generally was low and adult emergence near normal, with no noticeable delay in development, except at the two highest drop densities at the 50 mls/l concentration. Here only about 20% of the adults had emerged after 16 days, compared with untreated leaves. However, larval survival and adult emergence on some untreated leaves was also low, so no conclusions can be drawn from these data. These results, as with the "wet" applications described above, showed no significant translaminar activity.



In this, as well as subsequent drop generator experiments (data not shown), increasing drop size and/or density (=increasing dose) resulted in higher mortality. In two experiments to determine whether drop size, density, or concentration was most important in Margosan's activity against GHWF, results were similar (Table 6). Mortality ranged from 34-58% (The logit analysis of 150um drop data from a previous experiment predicted 50% mortality). Changes in drop size or density did not affect mortality or emergence, suggesting that these effects were dose-dependent and indicates that reducing drop size (e.g. applying Margosan with ULV equipment would not necessarily result in using less a.i. This contrasts with studies measuring efficacy of pyrethroids against GHWF which showed that LD<sub>50</sub> values were reduced with decreasing drop size (Mboob, 1975; Adams et al., 1987).

Table 6. Effects of variation in drop size and density applying the same Margosan dose/cm<sup>2</sup> on greenhouse whitefly larval mortality and adult emergence.

Margosan conc.(ml/l)	Drop density (no/cm <sup>2</sup> )	Drop size(μ)	N	% Mortality	% Emergence
				21 Days	28 Days
35	179	100	81	48	11
35	104	120	27	37	11
35	53	150	83	35	30
35	31	180	79	49	14
35	22	200	72	54	15
35	17	220	68	35	18
35	11	250	53	34	9
118	53	100	64	34	34
68	53	120	55	58	9
35	53	150	53	36	9
20	53	180	43	54	7
15	53	200	64	48	17
11	53	220	84	52	19
8	53	250	37	32	24
Control			51	14	60

Results of the bioassay conducted to determine how many larvae were hit by spray drops are shown in Table 7. The highest percentage of "hit" first-instar larvae was 13% (150um drops and 150 drops/cm<sup>2</sup>). Therefore, compared with mortality recorded in the drop generator experiments, relatively few larvae were actually hit by spray drops. These data indicate that Margosan must redistribute after deposition on a leaf surface. For example, with 100um drops, only a very few GHWF larvae were actually contacted by a spray drop even at the highest densities, yet this was sufficient to cause a marked reduction in adult emergence (based on data in Table 5). These results are similar to those obtained with mite eggs (Munthali and Scopes, 1981).

Table 7. Percentage of whitefly larvae hit with different sizes of spray drops at different drop densities.

Drop Size	Drop Densities (no/cm <sup>2</sup> )	No. Drops	N	% Hit
100um	6	45	39	0
	12	101	63	0
	25	211	28	0
	50	550	94	6
	100	800	63	0
	150	923	45	4
	200	551	104	5
150um	6	58	45	0
	12	113	69	1
	25	224	45	9
	50	450	74	1
	75	607	83	6
	100	884	99	7
	150	1201	189	13
250um	3	30	44	0
	6	58	50	0
	12	115	108	4
	25	230	150	7
	50	440	80	9
	75	692	49	10
	100	907	75	12

## CONCLUSIONS

These experiments show that Margosan-O is effective in controlling GHWF, whether the population was bifenthrin-resistant or susceptible. Deposition of Margosan on abaxial leaf surfaces was necessary to obtain significant effects, although initial direct contact with sessile GHWF larvae was not required. Apparently, redistribution of the Margosan occurred after application. Margosan also had sublethal effects on GHWF, showing larval development and delaying adult emergence. Margosan's activity appeared to be directly related to dosage applied, regardless of spray drop size, concentration, or density.

## LITERATURE CITED

- Adams, A.J., M.R. Abdalla, I.J. Wyatt, and A. Palmer. 1987. The relative influence of the factors which determine the spray droplet density required to control the glasshouse whitefly, Trialeurodes vaporariorum. Aspects of Applied Biology 14:257-266.
- Duncan, D.B. 1951. A significance test for difference between ranked treatments in analysis of variance. *Va. J. Sci.* 2:171-189.
- Jacobson, Martin. 1986. The neem tree: natural resistance par excellence. *Proc. ACS Symposium Series: Natural Resistance of Plants to Pests*, Maurice B. Green and Paul A. Hedin, eds: 220-231.

- Knodel, J.J., H.G. Larew, and R.E. Webb. 1986. Margosan-O, a commercial formulation of neem seed extract, controls Liriomyza trifolii on chrysanthemums. J. Agric. Entomol. 3 (3):249-254.
- Larew, H.G., J.J. Knodel-Montz, R.E. Webb, and J.D. Warthen. 1985. Liriomyza trifolii Burgess) (Diptera: Agromyzidae) control on chrysanthemums by neem seed extract applied to soil. J. Econ. Entomol. 78:80-84.
- Lindquist, R.K., H.R. Krueger, and M.L. Casey. 1986. Use of neem extract as a systemic treatment for Liriomyza trifolii on greenhouse chrysanthemum. Proc. BCPC-Pests and Diseases: 271-276.
- Mboob, S.S. 1975. Preliminary assessment of the effectiveness of two droplet sizes of insecticide for the control of glasshouse whitefly, Trialeurodes vaporariorum (Westwood). Plant Pathology 24:158-162.
- Meisner, J., K.R.S. Ascher, and M. Zur. 1983. The residual effect of a neem seed kernal extract sprayed on fodder beet against larvae of Spodoptera littoralis. Phytoparasitica 11 (1):51-54.
- Munthali, David C. and Nigil E.A. Scopes. 1982. A technique for studying the biological efficiency of small droplets of pesticide solutions and consideration of the implications. Soc. Chemical Industry: 60-62.
- Prabhaker, Nilima, N.C. Toscano, and D.L. Coudriet. 1989. Susceptibility of the immature and adult stages of the sweetpotato whitefly (Homoptera: Aleyrodidae) to selected insecticides. J. Econ. Entomol. 82 (4):983-988.
- Reynolds, K.M., L.V. Madden, D.L. Reichard, and M.A. Ellis. 1987. Methods for study of raindrop impact on plant surfaces with application to predict inoculum dispersal by rain. Phytopathology 77:226-232.
- Schmutterer, H. 1985. Which insect pests can be controlled by application of neem seed kernal extracts under field conditions? Z. Ang. Ent. 100:468-475.
- Steffens, R.J. and H. Schmutterer. 1982. The effect of crude methanolic neem (Azadirachta indica) seed kernal extract on metamorphosis and quality of adults of the mediterranean fruit fly, Ceratitis copitata Wied. (Diptera, Tephritidae). Z. Ang. Ent. 94:98-103.
- Zehnder, Geoffrey and J.D. Warthen. 1988. Feeding inhibition and mortality effects of neem-seed extract on the Colorado potato beetle (Coleoptera: Chrysomelidae). J. Econ. Entomol. 81 (4):1041-1044.

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# EFFECTS OF NEEM EXTRACTS ON BENEFICIAL INSECTS IN GREENHOUSE CULTURE //

K.A. Hoelmer, L.S. Osborne, and R.K. Yokomi

## ABSTRACT

Evaluation of selective pesticides, such as neem extracts, on beneficial insects is very important to their successful integration into pest management programs. Evaluation methodology is discussed. Results of tests with predators and parasitoids of cotton aphid and sweetpotato whitefly show that neem extracts were relatively non-toxic and did not greatly reduce predation and parasitism.

## INTRODUCTION

Many pesticides applied to crops for arthropod pest management have been relatively unselective, affecting not only the pest species but beneficial species as well. Beneficial organisms may be more sensitive than the target species to pesticides because of physiological, ecological, and behavioral differences. Due to the growing reluctance of consumers to accept produce treated with toxic pesticides, the increasing awareness that pesticide applications contribute significantly to groundwater contamination, the gradual loss of once-effective chemicals through development of resistance, and the increasing importance of integrated pest management in cropping systems, growers are under increasing pressure to seek alternative pest management tools.

Biological control of pests through the release of predators, parasitoids, and pathogens is one approach that can be used to avoid these problems; another is the development and use of new, highly selective pesticides active against specific types of pests but with reduced or no activity against non-target organisms.

Integrating chemical, biological and cultural methods of pest management into IPM programs for specific cropping systems is made easier with the availability of highly selective pesticides with minimal side effects. The availability of selective chemicals is often essential to the successful development of IPM programs (van Lenteren and Woets, 1988), especially in greenhouses where unsprayed refugia for natural enemies are usually not available. This is partly responsible for interest in pesticidal products derived from the neem tree, Azadirachta indica, such as neem seed kernel extracts and commercial formulations containing azadirachtin.

Although a great deal of information is available relating the effect of neem extracts on pest species, there is less data about impact on beneficial species, especially as it relates to factors other than direct toxicity. Published studies to date are discussed in a recent review by Schmutterer (1990). Neem extracts are not highly toxic to most types of predators and parasitoids, although some exceptions have been reported.

Neem extracts have many different types of effects on insects. These effects may be categorized as behavioral, reproductive, and

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Hoelmer and Yokomi are research entomologists, USDA-ARS, Horticultural Research Laboratory, Orlando, FL 32803; and Osborne is an associate professor of entomology, University of Florida, IFAS, CFREC, Apopka, FL 32703.



fitness modifiers. Beneficial insects need to be examined for these effects in addition to direct mortality. Significant sublethal effects from pesticide residues have been reported that can affect many different types of insect behavior (Haynes, 1988). Some examples of undesirable effects on beneficials include reduced response to pheromones or kairomones, altered rate of movement, dispersal from treated areas, disruption of molting, reduced oviposition or sterility, and reduced longevity. Desirable side effects, such as increased longevity, have also been reported (Joshi et al., 1982) although such effects should be examined carefully as they could be accompanied by other effects.

There is a continuing need to increase the taxonomic range of beneficial species tested with neem extracts. This is true not only for natural enemies, but also for pollinators and organisms of no economic but significant ecological value. Ideally, initial screenings of beneficials should be planned according to currently accepted standard testing protocols (Hassan, 1985; Hoogscarpel and Jobsen, 1984). These protocols have been designed to assess reductions in effectiveness of predators and parasitoids under conditions which reproduce the maximum exposure they are likely to receive in the field. Tests are hierarchical and can quickly identify pesticides that have no effect or are relatively harmless by measuring reductions in oviposition or parasitism. Further studies with neem extracts are also needed to focus more attention on possible long-term effects on the behavior and fitness of natural enemies, on their effectiveness as predators and parasitoids, and on the development and fitness of their offspring.

#### EVALUATION METHODS AND RESULTS

We conducted impact studies using Margosan-O, supplied by W. R. Grace Corporation. This formulation contained 0.3% azadirachtin. Test plants used included a variety of ornamental foliage and flowering plants. Laboratory tests were conducted at 25° C; greenhouse trials were conducted with daily temperatures fluctuating between 19 and 32° C. In all tests the formulation was diluted 1:150 with water.

The beneficials evaluated included: Delphastus pusillus, a coccinellid predator of Bemisia tabaci eggs, immatures and adults; an unidentified species of Scymnus, a coccinellid aphid predator established on greenhouse populations of Aphis gossypii and Myzus persicae; two parasitoids attacking Aphis gossypii, Lysiphlebus testaceipes (Aphidiidae) and Aphelinus asychis (Aphelinidae); and three parasitoids of sweetpotato whitefly, Eretmocerus californicus, Encarsia formosa, and Encarsia transvena (all aphelinids).

A quick screening was done by dipping hibiscus foliage in the diluted neem formulation. Once foliage had dried, adult beetles and parasitoids were confined (either individually, in groups of 2 to 3, or in male-female pairs) in petri plates or glass vials containing treated leaf discs or leaves. Controls were given untreated foliage. Parasitoids were fed by placing honey-soaked strips of tissue on the treated foliage. Beetles were fed before confinement but were not given food during this test. Treatment and control material was held in the laboratory and observations were made at regular intervals (1, 2, 4 hours post-exposure, and daily thereafter) to record mortality.



No Delphastus or Scymnus mortality was observed in control or treatment exposure arenas for two weeks, therefore the treatment had no immediate toxic effect on these coccinellids. In other tests, females fed treated Bemisia eggs for several days continued to oviposit. The normal life span of adult Delphastus is 2 to 3 months. Extended studies of longevity of treated beetles and the fate of their progeny are in progress.

Parasitoids are generally not as long-lived as coccinellids; in ventilated glass vials, survival of untreated controls decreased sharply during the first 24 to 48 hours even when wasps were provided with a food and water source. Some differences in survival were noted, however: the survival curves of Eretmocer were the same on treated and untreated foliage; whereas the aphid parasitoids, Lysiphlebus and Aphelinus, were slightly more sensitive to treated surfaces. Eretmocer male and female pairs confined in higher humidity sealed petri dishes with treated and untreated foliage survived without any mortality for 5 days. From these results it was concluded that neem extracts were not highly toxic to parasitoids.

The survival of treated, unemerged parasitoids within mummified hosts was assessed by dipping foliage with aphid or whitefly mummies in the azadirachtin formulation, or by dipping slides with double-stick tape containing parasitized whitefly scales. The Eretmocer emerging from one of these tests were retained separately to record longevity.

The emergence of Lysiphlebus adults from mummies was the same for treated and untreated foliage. Results of dipped whitefly scales indicated that Encarsia formosa and En. transvena emergence was the same from treated and untreated slides, but emergence of Eretmocer from treated slides was less than half that from untreated slides. Whiteflies parasitized by Eretmocer are penetrated from below by the larval parasitoid, leaving an opening that may allow the oily formulation to reach the developing adult parasitoid. In fact, parasitoids that never emerged seemed to be enveloped in more fluid than healthy ones which later emerged. Encarsia species develop entirely inside the whitefly following oviposition and cause no such wound. Because the tape used to fasten the scales to the slide appeared to retain more formulation than did treated foliage, Eretmocer was tested again by dipping parasitized scales still attached to foliage. Emergence of parasitoid adults was slightly but significantly lower from treated foliage (fig. 1).

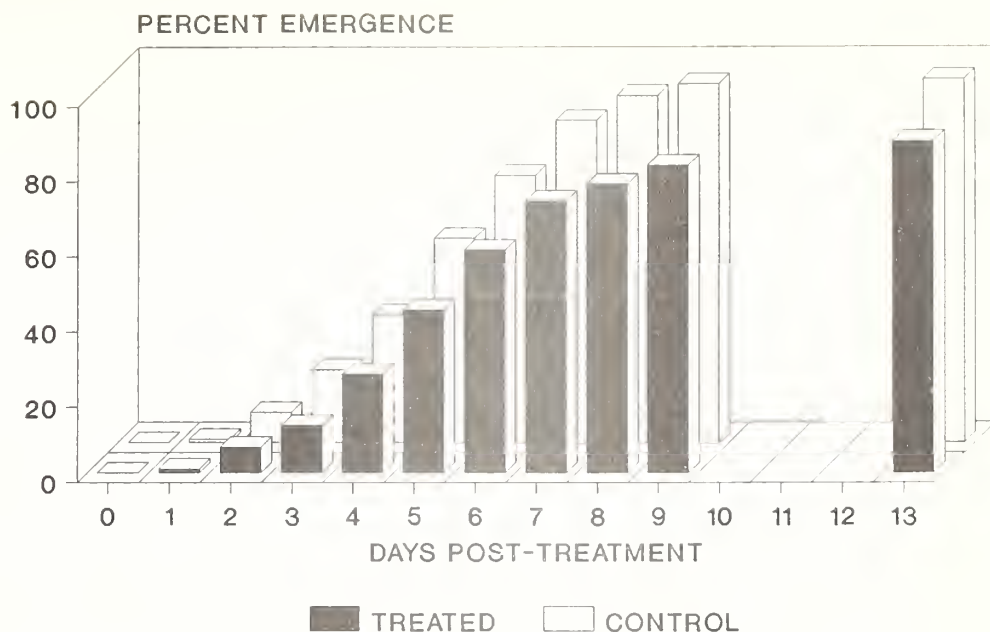


Fig. 1. Eretmoceris emergence (adult)

The mean longevity of those adults that successfully emerged was significantly greater for treated foliage compared with untreated foliage (fig. 2). The reason for this is not clear, although one possible explanation is that the treatment eliminated the least fit individuals, while those with higher fitness completed development and emerged. Further studies of progeny are underway.

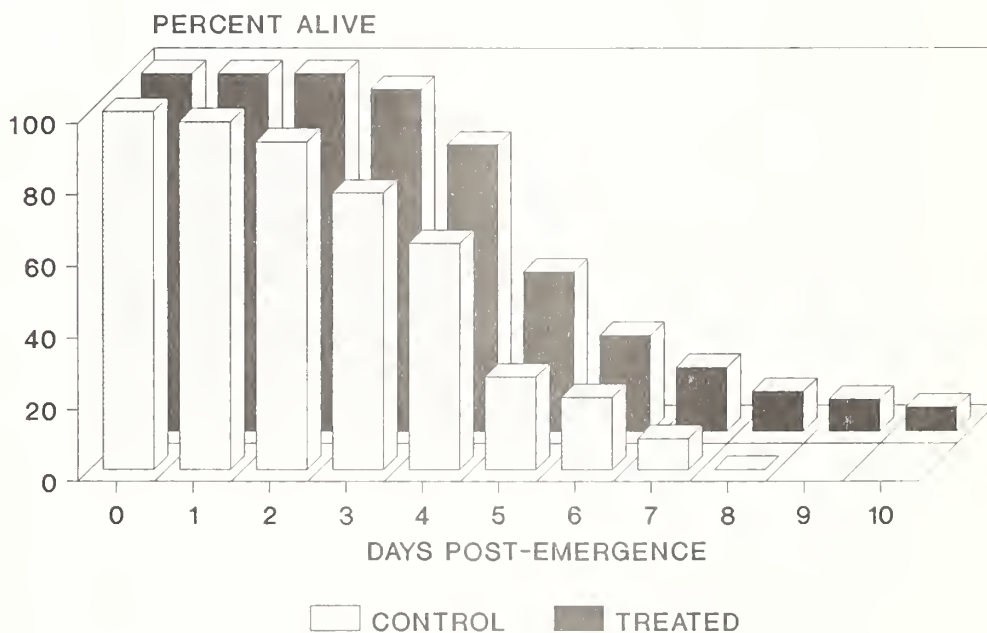


Fig. 2. Treated Eretmoceris F1 Longevity

The influence of treated prey or hosts on acceptance by natural enemies was examined with choice tests. Well-fed female *Delphastus* adults were placed individually in small petri plates and offered a number of leaf discs. The total number of eggs on all discs given to each beetle represented more than a typical adult's daily diet, when fed to satiation. Each disc contained only a fraction of this total, forcing beetles to inspect a number of discs to eat a full daily diet of eggs. The following day, the number of eaten and uneaten eggs was determined. This procedure was repeated an additional day. The results (fig. 3) indicate that *Delphastus* apparently preferred untreated eggs when given a choice the first day, but none of the beetles tested chose to feed to satiation. On the second day, this avoidance broke down and all of the beetles ate a greater percentage of eggs, including more of the treated eggs.

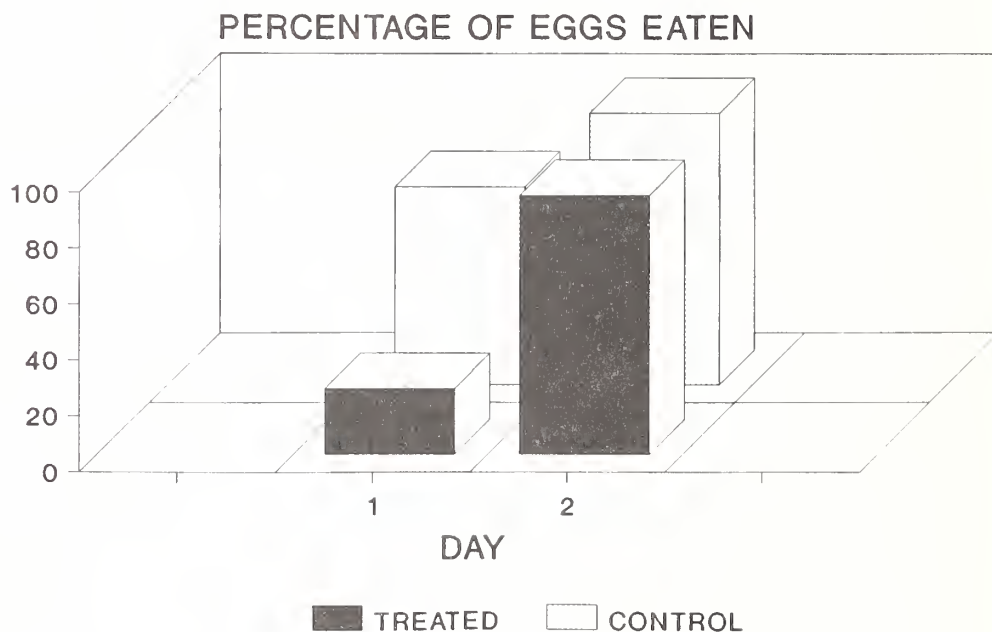


Fig. 3. *Delphastus* feeding preference

Preference by *Eretmocerus* was tested in a similar fashion by offering individual females a series of leaf discs with treated or untreated immature whiteflies. After 24 hours of exposure, the number of eggs deposited under the whiteflies were counted. Untreated whiteflies were attacked over three times as often as treated ones. The experiment was varied by placing equal numbers of treated and untreated tip-cuttings of hibiscus with whitefly immatures together in a small cage with a number of adult parasitoids. After three days, the number of whiteflies attacked was determined. Again untreated whiteflies were attacked 3:1 over treated ones. When parasitoids were caged with paired treated and untreated *Salvia* for 2 weeks, however, the ratio of untreated to treated whiteflies attacked declined, ranging from 1:1 and 2:1. This suggests that any avoidance of treated hosts or surfaces diminishes with time or lack of choice.

Parasitism by Eretmocerus and Encarsia transvena was recorded in several greenhouse trials in which Margosan-O was applied up to four times. In each case, plants treated with Margosan-O had levels of parasitism comparable to unsprayed controls or significantly higher than when other pesticides were used.

These results show that although neem extracts were not entirely without adverse effect on the beneficial species we tested, these effects diminished with time and did not prevent significant predation or parasitism. Longer term studies of possible effects on the reproduction and fitness of natural enemies are needed, but these preliminary results suggest that neem extracts are essentially compatible with the natural enemies.

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#### REFERENCES

- Hassan, S. A. 1985. Standard methods to test the side-effects of pesticides on natural enemies of insects and mites developed by the IOBC/WPRS Working Group "Pesticides and Beneficial Organisms". Bull. OEPP/EPPO Bull. 15:214-55.
- Haynes, K. F. 1988. Sublethal effects of neurotoxic insecticides on insect behavior. Ann. Rev. Entomol. 33:149-68.
- Hoogcarspel, A. P., and J. A. Jobsen. 1984. Laboratory method for testing side-effects of pesticides on Encarsia formosa Gahan (Hym., Aphelinidae). Results with pesticides used in glasshouse tomato crops in the Netherlands. Z. ang. Ent. 97:268-78.
- Joshi, B. G., G. Ramaprasad, and S. Sitaramaiah. 1982. Effect of a neem seed kernel suspension on Telenomus remus, an egg parasite of Spodoptera litura. Phytoparasitica 10(1): 61-3.
- van Lenteren, J. C. and J. Woets. 1988. Biological and integrated pest control in greenhouses. Ann. Rev. Entomol. 33:239-269.
- Schmutterer, H. 1990. Properties and potential of natural pesticides from the neem tree, Azadirachta indica. Ann. Rev. Entomol. 35:271-97.

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EFFECTS OF NEEM SEED EXTRACTS ON TEPHRITID FRUIT FLIES  
(DIPTERA: TEPHRITIDAE) AND THEIR PARASITIDS IN HAWAII

J. D. Stark, R. I. Vargas, and T. Y. Wong

ABSTRACT

The effects of a highly concentrated neem-seed extract (NSE) on metamorphosis, longevity, and reproduction of Mediterranean fruit fly Ceratitidis capitata (Wiedemann), oriental fruit fly Dacus dorsalis Hendel, and melon fly Dacus cucurbitae Coquillett were determined. Development of fruit fly parasitoids in azadirachtin-treated fruit flies was also examined. The potential use of NSE in integrated control strategies for tephritid fruit flies is discussed.

INTRODUCTION

The Mediterranean fruit fly, Ceratitidis capitata (Wiedemann); melon fly, Dacus cucurbitae Coquillett; and oriental fruit fly, Dacus dorsalis Hendel; are important economic pests in Hawaii and other areas of the world. These pests threaten the agricultural industry of California through recurrent introductions. The state of California has initiated five eradication programs for C. capitata in the last ten years.

A great deal of fruit fly control research has centered on eradication of these species. However, in Hawaii eradication has not been attempted and farmers still incur losses to fruit flies. Integrated pest management (IPM) programs that reduce populations of these pests are much needed in Hawaii and other areas of the world.

Because these fruit fly species pupate in soil, Shaw and Riviello (1961), Mohamad et al. (1979), and Saul et al. (1983), proposed the use of soil treatments with insecticides to target the vulnerable late third-instar larval and pupal stages as well as emerging adults to reduce or eliminate fruit fly populations from orchard areas. Presently, soil treatments with diazinon are used under fruit trees in California as one component of an overall eradication program when incipient introductions of fruit flies are detected. However, environmental concerns about the use of synthetic pesticides make it necessary to search for environmentally acceptable methods of pest control. Therefore, the use of neem-seed extracts (NSE) and neem-seed chemicals for insect control has received a great deal of attention in recent years.

Some studies have examined the effects of NSE on tephritid fruit flies. Singh and Srivastava (1983) found that crude neem-seed extracts deterred oviposition of D. cucurbitae and D. dorsalis. Steffens and Schmutterer (1982) determined that crude methanolic extracts of neem seeds exhibited a wide range of deleterious effects upon C. capitata that were fed the extract as larvae mixed with an artificial diet.

Larew et al. (1985) found that 0.4% crude NSE applied as a soil drench caused 100% inhibition of adult eclosion of Liriomyza trifolii (Burgess).

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Stark is a research entomologist, (USDA-ARS), P.O. Box 1330, Kapaa, Hi 96746. Vargas and Wong are research entomologists, USDA-ARS, P.O. Box 2280 Honolulu, Hi 96804



Azadirachtin is also known to retain its biological activity after exposure to ultraviolet radiation (UVR) thus making it a good potential soil insecticide. Barnby et al. (1989) found that azadirachtin retained its biological activity against pupating Heliothis virescens (F.) after 90 hours of continuous exposure to UVR and retained most of its activity after 200 hours of constant exposure to UVR. Therefore, C. capitata, D. dorsalis, and D. cucurbitae may be vulnerable to NSE applied as soil drenches and applications of NSE to soil in orchards may be an effective means of control for these species.

This study was conducted to determine whether NSE would be effective as a soil treatment for control of C. capitata, D. cucurbitae, and D. dorsalis.

#### MATERIALS AND METHODS

**Insects.** Fruit flies were obtained from mass-rearing stock maintained at the USDA Tropical Fruit and Vegetable Research Laboratory in Honolulu, Hawaii.

**Emergence of Adults Exposed as Larvae and Pupae.** Mature third-instar larvae were placed in containers of beach sand as they left an artificial diet. The sand was treated with various concentrations of a highly purified and concentrated neem-seed extract (NSE) supplied by W. R. Grace & Co., Columbia, Maryland. The NSE contained 7.3 g azadirachtin/l of ethanol and was devoid of other biologically active neem seed chemicals. The azadirachtin content of the NSE was determined throughout the course of the study by submitting subsamples of the concentrations used for each replicate to W. R. Grace & Co. for analysis by HPLC.

Seven days after the introduction of larvae into treated sand, the pupae were removed and were placed in containers with untreated sand. This step was added to ensure that emerging adults did not come into contact with azadirachtin, so that effects found in emergent adults could be attributed to exposure as larvae and pupae only.

Newly emerged adults were maintained on a 3:1 mixture of sugar and ICN enzymatic yeast hydrolysate (U.S. Biochemical, Cleveland, Ohio), and water.

The longevity of adults that emerged from treated sand was recorded daily for 21 days to determine whether there were differences in survival due to treatments. The entire study was replicated three- to eight-times.

**Effects on Fecundity, Fertility and Viability of F1 Progeny.** Adults that emerged from the treated sand at several concentrations were separated into groups of 10 females and 10 males and placed in containers with water and sugar-protein hydrolysate (3:1). The number of eggs oviposited (fecundity) at each treatment level was determined by the method of Albrecht and Sherman (1987). Three eggging dates were chosen which provided data throughout the reproductive period of each species (10, 15, and 20 days after adult emergence for D. dorsalis and D. cucurbitae; and 5, 10, and 15 days after emergence for C. capitata). An egg laying apparatus (Albrecht and Sherman, 1987) was introduced into each container and the number of eggs laid was determined 24 hours later on each eggging date. The percentage of eggs hatching (fertility) at each eggging date was determined 72 hours after the eggs were laid. The viability of F1 progeny was also determined.

Statistical Analysis. Concentration-emergence regressions were estimated by probit analysis (SAS Institute 1982). Differences between adult emergence from various treatments were considered significant when fiducial limits did not overlap. Longevity, fecundity, and fertility data were analyzed with analysis of variance (ANOVA) and means were separated by the Duncan option ( $P \leq 0.05$ ) (SAS Institute 1982). All percentage data were transformed to arcsine /proportion before analysis of variance.

## RESULTS

Soil Treatments. *D. dorsalis* and *D. cucurbitae* were significantly more susceptible than *C. capitata* to azadirachtin at EI95 (dose at which 95% of adult eclosion is inhibited) (Table 1). However, there were no significant differences in susceptibility to azadirachtin between the two *Dacus* species (Table 1). At the concentrations tested to determine 95% adult eclosion inhibition, pupation was not affected by azadirachtin and no outwardly visible differences in puparia were present. All larvae pupated but azadirachtin exhibited a dose-dependent, suppressive effect upon adult eclosion. Puparia from which adults failed to emerge were held for 1 month after adults had emerged from controls to determine if delayed emergence would occur. Prolonged developmental periods in insects treated with azadirachtin and NSE are common (Sharma et al. 1980, Warthen and Uebel 1982, Redfern et al. 1982, Ladd et al. 1984, Barnby et al. 1989). However, delayed emergence did not occur in the tephritids tested in this study and thereafter, pupae were dissected 1 or 2 days after adults emerged from controls. Upon dissection it was found that approximately 95% of the puparia at all concentrations tested contained living adults and they appeared morphologically the same as control flies. However, the treated flies could not expand their ptilina or wings and their legs exhibited no movement and they eventually died.

Table 1  
Eclosion inhibition (EI) of Tephritid fruit flies exposed to azadirachtin treated soil.

Species	n	Slope $\pm$ Std Err	EI (95% FL) 95 (ppm)
<i>C. capitata</i>	2820	2.87 $\pm$ 0.03	8.58 (7.08 - 11.10)
<i>D. cucurbitae</i>	2190	3.52 $\pm$ 0.13	3.95 (3.18 - 5.80)
<i>D. dorsalis</i>	2130	3.88 $\pm$ 0.03	5.37 (4.96 - 5.88)

Adult Longevity. Adults that emerged from treated third-instar larvae and pupae appeared morphologically normal but exhibited lower daily rates of survival than controls at the highest concentration level (Fig. 1). This phenomenon was most pronounced in *C. capitata* and *D. dorsalis* where survival was significantly lower in adults exposed to 5 parts per million (ppm) azadirachtin compared to controls.

Adult flies in our study exhibited no discernible symptoms of poisoning. However, the abdomens of surviving adults seemed to deflate over time, perhaps indicating that dehydration was occurring even though adults were observed feeding and drinking throughout their life-span.

Effects on Fecundity. Treatments with azadirachtin seemed to have no effect on the mating behavior of any of the species. C. capitata were observed mating as early as 2 days after eclosion while D. cucurbitae and D. dorsalis were observed mating as early as nine days after eclosion. Azadirachtin treatments had no significant effect on the fecundity of D. dorsalis and C. capitata. However, approximately 1.5 times more eggs were laid by D. dorsalis control flies than by flies treated with 2 ppm azadirachtin. A reduction in the number of eggs laid by C. capitata was evident at all treatment levels compared to controls but the reduction was not significant. Approximately 1.5 times more eggs were laid by C. capitata controls compared to C. capitata treated with azadirachtin. Significant differences were present between the number of eggs laid by treated D. cucurbitae and D. cucurbitae controls with the latter laying twice as many eggs as flies treated with 2 ppm azadirachtin.

Effects on Fertility. Azadirachtin treatments had no effect on egg hatch in these species. High levels of egg hatch occurred at all treatment levels and at all egg dates for each species.

Effects on Viability of Progeny. Treatments with azadirachtin had no detrimental effects on F1 larval or pupal development or adult emergence. F1 adults from treated parental stock did not differ from F1 adults from control parental stock.

## DISCUSSION

Steffens and Schmutterer (1982) found that C. capitata was very sensitive to crude methanolic neem-seed extracts (NSE) mixed with an artificial diet and fed to larvae. Although their extract probably contained chemicals other than azadirachtin it is likely that many of the effects they observed were due to azadirachtin. Significant reductions in pupation rate, adult emergence, pupal size, and longevity of adult C. capitata were present at concentrations as low as 15 ppm of the extract. Behavioral changes were also evident. Concentrations of NSE as low as 5 ppm significantly reduced startle activity, female olfactometric response and mating propensity. Flight ability was significantly reduced in C. capitata adults fed 10 ppm NSE as larvae.

Some of the results of our study are similar to those of Steffens and Schmutterer (1982). Dose-dependent reductions in adult emergence and longevity of emergent adults were evident. However, mating was not affected. Adult D. dorsalis and C. capitata treated with azadirachtin as larvae and pupae exhibited no significant reductions in fecundity and fertility. D. cucurbitae did exhibit a reduction in fecundity when treated with 2 ppm azadirachtin. It is unlikely that reduced fecundity found in D. cucurbitae was due to impairment of mating ability because these flies were observed to mate readily. Rather, the reduction in egg laying observed in D. cucurbitae was probably the result of physiological changes induced by exposure to azadirachtin. Flight ability and mating ability did not appear to be impaired in the flies we treated. Unlike the study of Steffens & Schmutterer (1982), we treated flies as late third-instar larvae and pupae while they treated feeding larvae. Moreover, they used an NSE while we tested azadirachtin. Differences between the two studies could account for the observed differences in the response of C. capitata.

The results of this study indicate that low concentrations of azadirachtin are effective inhibitors of adult emergence from treated soil. Furthermore, individuals that survive exposure as third-instar larvae and pupae die at much higher rates than controls. *D. cucurbitae* that survive treatments and emerge as adults also exhibit a reduced ability to lay eggs.

Soil insecticide treatments may be effective in suppressing tephritid populations. A soil treatment that selects for fruit flies while enabling parasitoid species to survive would be ideal. Since the majority of tephritid larvae exposed to azadirachtin pupated and living adults developed in puparia but were unable to emerge, we hypothesized that tephritids treated with azadirachtin may be able to sustain tephritid parasitoids (Braconidae: Hymenoptera). Schauer (1985) found that the spraying of aphid mummies with NSE did not prevent emergence of aphid parasitoids. Preliminary data in our lab indicates that fruit fly parasitoids can develop in azadirachtin treated fruit flies (J.D.S. unpublished data). Azadirachtin appears to be selective by inhibiting fruit flies from emerging to the adult stage while allowing parasitoids to emerge. Therefore, azadirachtin and NSE may be valuable as soil treatments used as one component of a fruit fly IPM program.

#### REFERENCES CITED

- Albrecht, C. P., & M. Sherman. 1987. Lethal and sublethal effects of avermectin b1 on three fruit fly species (Diptera: Tephritidae). *J. Econ. Entomol.* 80: 344-347.
- Barnby, M. A., R. B. Yamasaki, & J. A. Klocke. 1989. Biological activity of azadirachtin, three derivatives, and their ultraviolet radiation degradation products against tobacco budworm (Lepidoptera: Noctuidae) larvae. *J. Econ. Entomol.* 82: 58-63.
- Ladd, T. L., Jr., J. D. Warthen, Jr., & M. G. Klein. 1984. Japanese beetle (Coleoptera: Scarabaeidae): The effects of azadirachtin on the growth and development of the immature forms. *J. Econ. Entomol.* 77: 903-905.
- Larew, H. G., J. J. Knodel-Montz, R. E. Webb, J. D. Warthen, Jr. 1985. *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) control on Chrysanthemum by neem seed extract applied to soil. *J. Econ. Entomol.* 78: 80-84.
- Mohamad, R., Y. Ibrahim & S. Saud. 1979. Laboratory and field effectiveness of selected insecticides in preventing adult emergence of *Dacus dorsalis* Hendel (Diptera: Tephritidae). *Pertanika* 2: 47-50.
- Redfern, R. E., J. D. Warthen, Jr., E. C. Uebel & G. D. Mills. 1982. The antifeedant and growth-disrupting effects of azadirachtin on *Spodoptera frugiperda* and *Oncopeltus fasciatus*, pp. 129-136. In H. Schmutterer, K. R. S. Ascher & H. Rembold [eds.], Natural pesticides from the neem tree (*Azadirachta indica* A. Juss); Proceedings of the first international neem conference, Rottach-Egern 1980; German Agency for Technical Cooperation, Eschborn, Germany.
- SAS Institute. 1982. SAS user's guide: statistics. SAS Institute, Cary, N.C.



Saul, S. H., D. Tsuda, & T. T. Y. Wong. Laboratory and field trials of soil applications of methoprene and other insecticides for control of the Mediterranean fruit fly (Diptera: Tephritidae). J. Econ. Entomol. 76: 174-177.1983

Schauer, M. 1985. Die Wirkung von Nieminhaltsstoffen auf Blattläuse und die Rubenblattwanze. Ph.D. thesis. Univ Giessen. FRG.

Sharma, G. K., C. Czoppelt & H. Rembold. 1980. Further evidence of insect growth disruption by neem seed fractions. Z. Ang. Entomol. 90: 439-444.

Shaw, J. G. & M. S. Riviello. 1961. Exploratory studies with soil toxicants to control Mexican fruit fly. J. Econ. Entomol. 54: 666-668.

Singh, R.P. & B.G. Srivastava. 1983. Alcohol extract of neem (Azadirachta indica a. juss) seed oil as oviposition deterrent for Dacus cucurbitae (Coq.). Indian Journal Entomology 45: 497-498.

Steffens, R. J. & H. Schmutterer. 1982. The effect of a crude methanolic neem (Azadirachtin indica) seed kernal extract on metamorphosis and quality of adults of the Mediterranean fruit fly, Ceratitis capitata Wied. (Diptera, Tephritidae). Z. Ang. Entomol. 94: 98-103.

Warthen, Jr., J. D., & E. C. Uebel. 1982. Effects of azadirachtin on house crickets, Acheta domesticus. pp. 137-148. In H. Schmutterer, K. R. S. Ascher & H. Rembold [eds.], Natural pesticides from the neem tree (Azadirachta indica A. Juss); Proceedings of the first international neem conference, Rottach-Egern 1980; German Agency for Technical Cooperation, Eschborn, Germany.



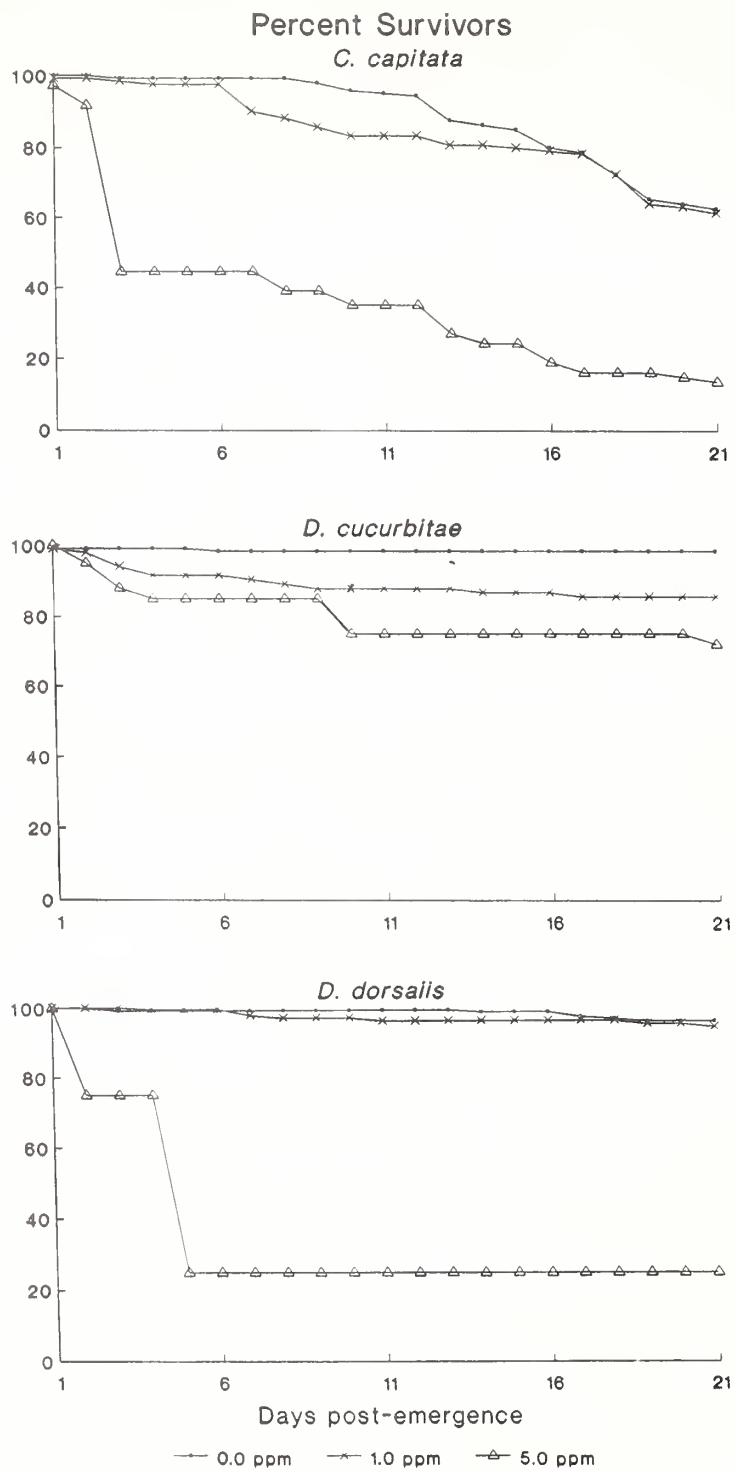


Fig. 1. Mean daily survival of emergent adult Tephritid fruit flies that survived treatment with azadirachtin as third-instar larvae and pupae.

## Novel Uses of Neem Components

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### TREATMENT OF CUTTINGS BEFORE SHIPMENT WITH NEEM

R. D. Oetting, K. C. Sanderson, and D. A. Smith

#### ABSTRACT

Chrysanthemum cuttings were treated by soaking the base of the cuttings or by vacuum infusion with extracts from neem seeds and a commercial formulation of azadirachtin. Both were effective in interrupting the development of Liriomyza trifolii (Diptera: Agromyzidae).

#### INTRODUCTION

There has been an increase in environmental concerns associated with synthetic insecticides and stimulated interest in natural chemicals. As a result, research has intensified on soaps, oils, and other natural products. One of these compounds that received attention was the extracts of neem seed (Azadirachta indica A. Juss. Gill and Lewis (1971) reported that the extract of neem beans had systemic activity against insects. Neem extracts have been tested against several insect species and had a broad spectrum of activity (Webb et al., 1983, Flint and Parks, 1989). Of special interest, was activity of neem against the leafminer Liriomyza trifolii (Burgess), a major pest of ornamental crops (Larew et al., 1985). Webb et al. (1983) found that neem extract was efficacious for leafminer larvae but foliage dipped in neem extract showed no adverse effects for adults. However, if adults laid eggs in treated leaves they failed to develop. L. trifolii was a pest of chrysanthemum, Dendranthema grandifolium Tevzel, and was especially troublesome because the larvae mine within leaf tissue and can easily be shipped around the world within the plant tissue when cuttings were sent to greenhouse plant growers. As a foliar spray, neem seed extract acted as a feeding inhibitor, an insect growth regulator or an insecticide against L. trifolii (Lindquist et al., 1986). However, it also had systemic activity against L. trifolii for 3 weeks when applied as a drench to the potting media (Larew et al., 1985). Foliage was protected from leafminer damage when roots were dipped in a neem solution (Larew, 1988) promoting the possibility that chrysanthemum cuttings might be treated before shipment and be protected until placed in pots at the growers range. Rooted cuttings were successfully protected following a root soak in solutions of permethrin (Lindquist et al., 1980). Lindquist et al. (1986) found that soaking cuttings of chrysanthemum in neem solutions reduced leafminer damage but plant growth was also effected. Experiments were conducted in Georgia and Alabama to determine if chrysanthemum cuttings could be treated and then shipped to another location for planting to protect the new plants against leafminer infestation (Sanderson et al., 1989). The treatment of chrysanthemum cuttings for protection against leafminers will be discussed in this paper.

Oetting is an associate professor, Dept. of Entomology, Univ. of Georgia, Georgia Agric. Experiment Station, Georgia Station, Griffin, GA 30223; Sanderson is a professor and Smith an associate professor, Dept. of Horticulture, Alabama Agric. Experiment Station, Auburn Univ., Auburn, AL 36849.

#### METHODS OF TREATMENT OF PROPAGATION MATERIALS

The treatment of propagation material for chrysanthemums in preparation for shipment or planting was primarily on the basal portion of the cutting. Basal soak applications were made by placing the cut end of the cuttings in a neem solution. The other methods were the treatment of the potting media containing the rooted cuttings and infusion of cuttings placed in a neem solution within a vacuum chamber.

##### Basal Soak

Larew (1988) soaked rooted cuttings of chrysanthemums cv. 'Iceberg' in 0.4% neem seed extract. Cuttings were left in the solution for 24 hours followed by a 1 min. dip in mild detergent (5ml Ivory liquid/liter H<sub>2</sub>O), in 20% ethanol for 1 min., and then in two 1-min water dips. The cuttings were then planted and leafminer damage evaluated for 4.5 weeks.

Lindquist et al. (1986) conducted a similar experiment with rooted cuttings of the same chrysanthemum cultivar. They investigated the effects of different concentrations (0.5, 0.15, 0.075, and 0.3%) of crude neem extract. The rooted cuttings were allowed to soak for 24 hours. In addition, they tested an emulsifiable concentrate formulation of 3000 ppm azadirachtin. Rooted cuttings were soaked in the azadirachtin utilizing concentrations of 0.1, 0.3, 1.0, and 3.0% at treatment times of 2, 4, and 24 hours. In their experiments the plants were not rinsed and were planted in potting media in pots and placed in a greenhouse. The plants were observed for four weeks.

##### In-transit Soak

Sanderson et al. (1989) investigated the feasibility of treating rooted cuttings of chrysanthemums cv. 'Iridon' and 'Tara' while in shipment. Rooted cuttings were packed in plastic bags (50 cuttings/bag) for shipment from the propagator. These cuttings had potting media on roots and the bottom of the plastic bags were solid with potting media around the roots. A 0.45% solution of a commercial formulation of azadirachtin (Margosan-OR) was poured into each bag, and the bags were sealed by folding for shipment. The solution was added to the level of saturation of the potting media. The Plants were then shipped (overnight) from Auburn, AL to Griffin, GA. When the plants arrived at their destination, they were removed from the shipping container and potted directly into potting media, without cleaning the treated soil from the roots, in pots and placed in a greenhouse. Plants were then exposed to leafminer adults and evaluated for leafminer infestation.

##### Vacuum Infusion

In addition to within-bag treatments of rooted cuttings, experiments were conducted on the feasibility of vacuum infusion of azadirachtin into rooted and unrooted cuttings of chrysanthemum cv. 'Manatee Iceberg' before shipment to the grower (Oetting and Sanderson, unpublished). In these experiments, cuttings were treated by vacuum infusion and compared to plants treated by basal

soaking for vacuum infusion cuttings were submerged in a tray containing the experimental solution and then placed in a vacuum chamber. Following treatment the cuttings were removed from the chamber and allowed to set in the solution for a few minutes, air dried, and prepared for shipment. Plants were then shipped (overnight) from Auburn, AL to Griffin, GA and planted in potting media and placed under greenhouse conditions for exposure to leafminers and evaluation of subsequent infestation.

## RESULTS AND DISCUSSION

Leafminer larval population, pupation, and adult emergence were monitored to determine if the treatments affected development and survival. There were significant reductions in leafminer populations in at least one concentration with each of the different treatment methods discussed.

### Basal Soak

Results of basal soak experiments with rooted chrysanthemum cuttings indicate that pupal and adult emergence from foliage of treated plants had significantly less leafminer emergence (Larew, 1988). However, the emergence was not reduced from the new foliage that developed after treatment. This would indicate that plants could be protected by basal soak but the protection would probably not last for more than a couple of weeks. The old leaves may be protected from leafminer infestation longer but the new leaves would support a leafminer population.

Basal soak of chrysanthemum rooted cuttings in crude extract solutions of neem did not affect the number of larvae in leaves (Lindquist et al., 1986). However, the percent larval survival was reduced, especially at higher concentrations. The azadirachtin was probably acting as a insect growth regulator and interfered with normal development. In addition, pupal survival was reduced at all levels of azadirachtin concentration. Lindquist et al. (1986) obtained similar results with the commercial formulation, a reduction in leafminer survival and plant growth. The pupation and adult emergence were affected the most and the higher the concentration and longer the duration of the soak the more significant the reduction in leafminer survival. Insecticidal effects disappeared after 2 to 4 weeks. This would still allow control of larvae present in rooted cuttings when shipped from the propagator to the grower. It could also protect chrysanthemums from the initial infestation of leafminers after placement of plants in the growing bay.

The treatment of chrysanthemums by basal soaking also affected the growth of the plants. The amount of growth reduction was directly related to the concentration and duration of soaking. The higher the concentration and longer the soak the greater the reduction in plant growth. This regulation of plant growth was temporary and the plants recovered in about 4 weeks. This reduction would not necessarily be negative for chrysanthemums grown in pots. In pot plant production chrysanthemums are normally treated with plant

growth regulators to reduce the height of plants and keep a more compact plant. As long as the phytotoxicity is reduction in height and not plant tissue damage such as deformation, chlorosis, or necrosis it would not be as critical. The production of chrysanthemums for cut flower production would be a different situation.

#### In-transit Soak

The azadirachtin-treated plants were 24 hours in-transit from Alabama to Georgia and were under mist for 24 hours before being exposed to a leafminer population. Then they were evaluated 2, 3, and 4 weeks after the in-transit treatment. Leafminer populations were not significantly reduced until the fourth week after treatment. In the fourth week the adult emergence was significantly reduced. The populations of larvae and pupae from the neem-treated plants were not significantly different from water treated plants during the duration of the test. However, populations were low in the test area and leafminer behavior in the area indicated that the leafminer adults were repelled from coming into the test area. Larval counts were similar in each of the three weeks evaluated. Even though the populations of leafminers were not large enough to get significant reductions, there was a trend for reduction in pupation and adult emergence in the azadirachtin-treated plants. The results of the potting media treatment were similar to those received with the basal soak indicating either method would result in disruption of the life cycle and reduce the chance of reinfestation from subsequent L. trifolii generations.

#### Vacuum Infusion

In the experiments which compared the use of basal soak and vacuum infusion of rooted and unrooted cuttings with different concentrations of azadirachtin, both methods significantly reduced the pupation and subsequent adult emergence. In both the soak and infusion treatments there was a concentration response with greater reduction of pupation and adult emergence with increased concentrations of azadirachtin. None of the treatments resulted in an effect on larval mining in the chrysanthemum leaves. The effect on the leafminer population disappeared 3-4 weeks after treatment.

Plant damage was also observed following vacuum infusion at the highest concentration of azadirachtin (27 ppm). The damage was reduction of growth and slight necrosis and cupping of leaf margins. One treatment was short soak (2 hrs) with a high concentration of azadirachtin (270 ppm). In this treatment there was severe distortion and stunting. The plants were shorter than all other treatments and the leaves were twisted with slight necrosis. These plants never recovered from the damage and had obvious damage at the conclusion of the experiment. This was the only treatment that did not recover after 3 or 4 weeks. It was also the treatment with the lowest adult emergence of leafminers in all tests.



## CONCLUSION

A review of the literature and our experiments indicate that there is a potential for neem extract as a propagation treatment before, during, or after shipment to reduce leafminer populations. The foliage present at the time of treatment was protected but subsequent plant growth was not. Treatment with azadirachtin did cause some plant damage, primarily expressed as reduced height. Since chrysanthemums grown for pot plant production are treated with plant growth regulators, this would not be detrimental as long as there was no distortion of plant tissue.

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## REFERENCES

- Flint, H. M. and N. J. Parks. 1989. Effect of Azadirachtin from the neem tree on immature sweetpotato whitefly, Bemisia tabaci, (Homoptera: Aleyrodidae) and other selected pests species on cotton. J. Agric. Entomol. 6(4): 211-215.
- Gill, J. S., and C. T. Lewis. 1971. Systemic action of an insect feeding deterrent. Nature (London) 232: 402-403.
- Larew, H. G. 1988. Limited occurrence of foliar-, root-, and seed-applied neem seed extract toxin in untreated plant parts. J. Econ. Entomol. 81(2): 593-598.
- Larew, H. G., J. J. Knodel-Montz, R. E. Webb, and J. D. Warthen. 1985. Liriomyza trifolii (Burgess) (Diptera: Agromyzidae) control on chrysanthemum by neem seed extract applied to soil. J. Econ. Entomol. 78: 80-84.
- Lindquist, R. K., H. R. Krueger and M. L. Casey. 1986. Use of neem extract as a systemic treatment for Liriomyza trifolii control on greenhouse chrysanthemum. Brit. Crop Prot. Conf.-Pests and Diseases-1986: 271-276.
- Lindquist, R. K., D. E. Simonet, and H. R. Krueger. 1980. Comparison of permethrin applied as a foliar spray and root soak for cabbage looper control on greenhouse chrysanthemum cuttings and celery transplants. J. Econ. Entomol. 73: 381-384.
- Sanderson, K. C., R. D. Oetting, and D. A. Smith. 1989. In-transit neem insecticide treatment of rooted chrysanthemum cuttings controls leafminer. HortScience 24(5): 856.
- Webb, R. E., M. A. Hinebaugh, R. K. Lindquist, and M. Jacobson. 1983. Evaluation of aqueous solution of neem seed extract against Liriomyza sativae and L. trifolii (Diptera: Agromyzidae). J. Econ. Entomol. 76: 357-362.

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NEEM LEAF EXTRACTS INHIBIT AFLATOXIN BIOSYNTHESIS IN ASPERGILLUS FLAVUS AND A. PARASITICUS

Deepak Bhatnagar, Hampden J. Zeringue Jr. and Susan P. McCormick

# ABSTRACT

Aflatoxins are carcinogenic secondary metabolites of the fungi Aspergillus flavus and A. parasiticus. The effect of neem (Azadirachta indica) leaf extracts on Aspergillus growth and aflatoxin biosynthesis was investigated. The extracts were prepared by blending 100g (wet weight) of fresh leaves in 1 liter of 10mM potassium phosphate (pH 7.0). The formulation (1 to 50%, vol/vol), when added to fungal growth medium prior to inoculation, did not affect fungal growth but essentially blocked aflatoxin biosynthesis in both A. flavus (100%) and A. parasiticus (>95%) at concentrations greater than 10% (vol/vol). Injection of the neem leaf extract followed by an A. flavus spore suspension (48 hrs later) onto the surface of locks of developing cotton bolls (30-day post anthesis) did not affect fungal growth in the bolls, but the seeds from the locules exhibited >98% inhibition in aflatoxin production. In vitro studies with the fungi suggest that non-volatile neem leaf constituents inhibit aflatoxin biosynthesis in the early stages of the biosynthetic pathway. Practical applications of this observation are being developed to eliminate the preharvest contamination of crops with aflatoxin.

# INTRODUCTION

Aflatoxins are biologically active, secondary metabolites produced by the fungi, Aspergillus flavus Link ex. Fries and A. parasiticus Speare (Detroy et al, 1971). These toxins have received considerable attention because they have the characteristic of being the most carcinogenic of all known natural compounds (Groopman et al. 1981) and several million dollars worth of crops are declared unfit for consumption every year due to aflatoxin contamination. Aflatoxin contamination can occur due to infection and growth of aflatoxin-producing fungi in crops before harvest (Detroy et al, 1971). Several conventional methods have been examined to manage preharvest aflatoxin contamination (Widstrom, 1987; Zummo and Scott, 1989; Zaika and Buchanan, 1987). These control methods have reduced but not eliminated preharvest aflatoxin contamination in corn, peanuts and cottonseed. Novel biotechnological strategies are being developed (Bhatnagar et al, 1989; Cleveland et al, 1990), to be used in conjunction with conventional methods, to address this serious problem.

Azadirachta indica Juss. (syn. Melia azadirachta L.) commonly known as "margosa" "neem" and "nim" is an ornamental tree of Asia and Africa. Neem components have reputed value for their medicinal, antimicrobial, antinematode and insect repellent properties (Singh et al, 1980; Jacobson, 1989).

Owing to the antimicrobial properties of the neem plant, the current study was conducted to assess the inhibitory effect of aqueous extracts of neem leaves on A. flavus and A. parasiticus growth and aflatoxin production in submerged fungal cultures as well as in developing cotton bolls. The conditions which favor inhibition of aflatoxin synthesis and the mode of action of this inhibition were also examined.

Bhatnagar is a Research Geneticist, USDA-ARS, Southern Regional Research Center, P. O. Box 19687, (New Orleans, LA) 70179; Zeringue is a Research Chemist at the same Center; McCormick is presently a Research Chemist, USDA-ARS, Northern Regional Research Center, Peoria, IL.

## MATERIAL AND METHODS

Preparation of neem leaf extracts. Fresh neem leaves were obtained from the USDA-ARS Subtropical Research Station, Miami, Florida. After washing the leaves thoroughly with sterile distilled water, extracts were prepared by blending 100 g (wet weight) of fresh leaves in 1 liter of sterile distilled water or 10 mM potassium phosphate buffer (pH 7.0). Extracts were filtered through several layers of cheesecloth, and the filtrate was centrifuged for 15 min at 7,000 xg. The pellet was discarded and the supernatant was stored at 4°C. Soluble extracts were sterilized by passing through a Millipore filter (0.22 µm pore size). In addition, in some cases, a portion of the leaf formulations were autoclaved to achieve sterilization.

Fungal cultures. The fungal strains used in this study were wild-type aflatoxigenic strains of *A. flavus* (SRRC 1100) and *A. parasiticus* (SRRC 143). The cultures were maintained on PDA plates. The spores of the fungi were harvested after a 7 day incubation at 28°C and a spore suspension (10<sup>8</sup> spores/ml) was prepared in sterile, distilled water containing 1% Triton X-100. These spore suspensions were used for inoculation of both liquid culture media and cotton bolls.

Fungal growth in submerged cultures. Fungal growth was determined in 250-ml flasks containing 100 ml of growth medium (GM) (Adye and Mateles, 1964), 0.1 ml of the spore suspension, and 0 to 40% vol/vol neem leaf formulations. The flasks were incubated for 2-4 days on a shaker incubator (Lab-Line Instruments, Inc.) at 150 rpm and 28°C. Growth of the fungus was recorded after four days by harvesting the mycelial pellets on oven-dried Whatman No. 42 filter papers. The filter papers containing the fungal mycelial mass were oven-dried at 70°C for 24 hr, and the dry weight of the fungus was determined as an index of fungal growth. For the study of the conversion of secondary metabolites to aflatoxins in the presence of neem extract, 1g of wet mycelia were washed thoroughly and transferred to a low sugar replacement medium (LSRM) as described earlier (Bhatnagar et al, 1987).

Fungal growth conditions in cotton bolls. Developing cotton bolls, thirty days post anthesis, were obtained from approximately 4 month old Acala SJ-2 cotton plants growing under greenhouse conditions. Two 3 mm holes were produced on the surface of all treated bolls, to simulate the exit holes of the pink boll worm, *Pectinophora gossypiella*, one of the main sources of *A. flavus* cotton boll infection (Lee et al, 1987). All inoculations were made through these surface holes. The holes were produced approximately 1 mm from one side of the exterior capillary suture line. This position allowed the treatment area to be located in the center of the total surface area of the developing fibers and, therefore, confined the treatment to an individual lock. Ten µl of *A. flavus* spore suspension and 50 µl neem leaf extract, each inoculated in separate bored holes, were used in the treatments. The controls included inoculations of 10 µl *A. flavus* spore suspension and 50 µl sterile, distilled water. The developing bolls were harvested 13 days after inoculation. The seeds in the treated locks were delinted and extracted for aflatoxin B<sub>1</sub>.

Cottonseeds were separated from either neem leaf extract and *A. flavus* inoculated or *A. flavus* alone inoculated locks to assay for fungal growth within the seeds. These seeds were acid delinted with concentrated sulfuric acid (1 min), rinsed in distilled H<sub>2</sub>O, treated in 10% clorox (1 min) and rinsed several times with sterile, distilled H<sub>2</sub>O. The surfaced sterilized seeds were positioned on PDA plates and after incubation for 4 days at 28°C the radial growth of *A. flavus* mycelia was compared to radial growth from control seeds.

Extraction and assay of metabolites and aflatoxins. After incubation in submerged cultures, mycelial pellets and the medium were extracted with aqueous acetone, followed by methylene chloride (Bhatnagar et al, 1987). The aflatoxins were extracted from delinted cottonseed according to established procedures (AOAC, 1984).

Aflatoxins were separated on silica gel thin layer chromatographic (TLC) plates in ether:methanol:water (96:3:1, vol/vol/vol). The toxins were quantitated by comparison of fluorometric scans (360 nm) of TLC plates containing the extracted samples with aflatoxin standards run on the same plate (Bhatnagar et al, 1987). Aflatoxin precursors from the mycelia were identified with HPLC by the procedure described by McCormick et al (1988).

Determination of volatile components of neem leaves. Volatiles from neem leaf extracts were collected onto Tenax GC (60-80 mesh) tubes as described earlier (Bhatnagar and McCormick, 1988). The volatiles absorbed on the Tenax tubes were heat desorbed onto a capillary chromatographic column and were analyzed with a Finnigan MAT GC/MS 4000 system interfaced with an external closed inlet device (Bhatnagar and McCormick, 1988). Data acquisition and analysis were accomplished with a Finnigan-Incos data system and the compounds were identified on the basis of computer-assisted library searches.

Effect of volatile components of neem leaves. Activity of volatiles from the blended neem leaf extracts on growth and aflatoxin production in *A. flavus* was determined on PDA agar plates (Fig. 1) by the procedure described earlier (Bhatnagar and McCormick, 1988). The radial growth was recorded each day over a four-day test period. At the end of the four days, Petri plate contents were extracted for aflatoxin analysis.

## RESULTS

Effect of neem leaf formulations on Aspergillus growth and aflatoxin production in submerged cultures. Neem leaf extracts (0 to 40%, vol/vol) did not affect fungal growth (i.e. mycelial dry weight), but essentially blocked (>95%) aflatoxin synthesis at concentrations greater than 15% (vol/vol) (Table 1, Fig. 2). The effect of the neem leaf extracts was more pronounced in *A. flavus* than in *A. parasiticus*; total inhibition of aflatoxin synthesis was not achieved in *A. parasiticus*.

TABLE 1  
Effect of neem leaf extracts on *A. flavus* and *A. parasiticus* growth in submerged cultures<sup>a</sup>

Concentration of extract (vol/vol) <sup>b</sup>	Mycelial dry weight (g) <sup>c</sup>	
	<i>A. flavus</i>	<i>A. parasiticus</i>
0	0.96 ± 0.15	1.11 ± 0.16
5	0.94 ± 0.11	1.04 ± 0.21
10	0.98 ± 0.09	1.19 ± 0.12
25	1.09 ± 0.18	1.08 ± 0.10
40	1.01 ± 0.11	1.13 ± 0.22

<sup>a</sup> The results are the means and standard errors of 4 experiments with 2 replicates each.

<sup>b</sup> The neem extracts were prepared as described in Materials and Methods.

<sup>c</sup> The mycelial dry weight were determined after 4 days of fungal growth.



Effect of neem leaf formulations on *A. flavus* growth and aflatoxin production in cotton bolls. Treating developing cotton bolls (30 day post anthesis) with the neem leaf extract 48 hrs prior to infecting the bolls with a spore suspension of *A. flavus* produced almost total (>98%) inhibition of aflatoxin synthesis measured in cottonseed from treated cotton boll locks 13 days after spore inoculation (Table 2). Similar cotton bolls which received a simultaneous application of both *A. flavus* spore suspension and neem leaf extract treatment exhibited only a 16% inhibition of aflatoxin production in the cottonseeds after the 13 day incubation period.

When delinted, surface-sterilized seeds from control or treated locks were placed on PDA plates and the fungus was allowed to grow for four days, the radial fungal growth in the case of seeds from treated locks was 93-96% of that of controls (Table 2).

TABLE 2

Effect of neem leaf extracts on *Aspergillus flavus* growth and aflatoxin B<sub>1</sub> production in developing cottonseed<sup>a</sup>

Treatment	Aflatoxin B <sub>1</sub> /g seed (ng)	<i>A. flavus</i> growth <sup>b</sup> (cm)
<i>A. flavus</i> inoculated	38,692 ± 3,864	25.1 ± 0.53
<i>A. flavus</i> and neem extract inoculated at the same time	30,799 ± 13,397	24.0 ± 0.48
<i>A. flavus</i> inoculated, 48 hours after neem extract injection	8,435 ± 367	23.5 ± 0.71

<sup>a</sup> Assayed 13 days after fungal spore inoculation. The results represent the means and standard deviations of at least three experiments.

<sup>b</sup> 4-day radial growth of emerging *A. flavus* from seeds of treated locks.

Effect of neem leaf volatiles on fungal growth and aflatoxin production on agar. The content of 10 of 13 major volatile components was significantly higher in the blended neem leaf extract than in the autoclaved, heated extract (Bhatnagar and McCormick, 1988); the major volatile component being 3-methyl-2-buten-1-ol (Fig. 3).

The bioactivity of the neem leaf volatiles was assessed by measuring fungal growth and aflatoxin production by *A. flavus* grown on agar medium and exposed to an atmosphere containing volatiles from blended neem leaf extract (Bhatnagar and McCormick, 1988). Volatiles from blended neem leaf extracts did not affect either aflatoxin synthesis or fungal growth during incubation of the fungus on PDA plates (Table 3).



TABLE 3.  
Effect of volatiles in neem leaf extracts on fungal growth and aflatoxin production by *A. flavus*<sup>a</sup>

Duration of incubation	Treatment <sup>b</sup>	Aflatoxin B <sub>1</sub> produced (μg)	Fungal growth (cm)
2 days	Control	4.9 ± 1.5	2.8 ± 0.3
	Neem extract	3.8 ± 1.0	2.9 ± 0.4
4 days	Control	16.3 ± 2.0	4.8 ± 1.1
	Neem extract	17.1 ± 1.6	5.2 ± 1.3

<sup>a</sup> Fungal growth and aflatoxin production were determined as described in Material and Methods. The results represent the means and standard errors of 3 experiments.

<sup>b</sup> 1.5 ml of neem-leaf extract was added in the treated test and an equal volume of sterile-distilled water was added in the control test.

Localization of inhibition of aflatoxin biosynthesis by neem leaf extracts. Mycelia of *A. flavus* were grown for 2 days in GM containing 20% neem extract and the aflatoxin content measured after transferring 1g of mycelia to a low sugar resting medium in the presence of several aflatoxin precursors (Table 4). The results clearly indicate that although aflatoxin synthesis was inhibited by neem leaf extract, the affected fungal mycelia were able to convert aflatoxin precursors to aflatoxin B<sub>1</sub>. When the extracts of treated mycelia (with no aflatoxin precursor added) were analyzed for the presence of aflatoxin precursors by the HPLC procedure described earlier (McCormick et al, 1988), none of the metabolites detected in extracts of control mycelia were found in the treated extracts (data not shown).

TABLE 4  
Ability of *A. flavus* mycelia grown in the presence of neem leaf extract to utilize aflatoxin precursors.

Precursor added	Amount added (μg)	Aflatoxin B <sub>1</sub> produced in LSRM (μg) <sup>a</sup>	
		Control Mycelia	Neem-treated mycelia
none		8.3 ± 1.8	ND
norsolorinic acid	40	11.1 ± 2.6	3.6 ± 1.1
averantin	30	13.3 ± 1.9	5.9 ± 1.2
versicolorin A	20	17.4 ± 1.1	8.8 ± 2.2
sterigmatocystin	10	15.2 ± 2.1	6.4 ± 2.0
O-methylsterigmatocystin	10	17.6 ± 2.0	8.1 ± 1.1

<sup>a</sup> One-g fractions of wet 48-hr-old mycelia were obtained from fermentation carried out either in the absence (control mycelia) or presence (neem-treated mycelia) of 20% (vol/vol) of neem leaf extract in growth media. Ability of these mycelia to utilize aflatoxin precursors in resting media were determined as described in Material and Methods. The values represent the means and standard errors of two experiments with two replicates each.

## DISCUSSION

In an effort to obtain a botanically derived inhibitor of Aspergillus flavus or A. parasiticus growth and aflatoxin production by these fungi, the efficacy of extracts of neem leaves were investigated in our laboratory (Bhatnagar and McCormick, 1988). Aqueous extracts of neem leaves, when added to fungal growth media prior to fungal spore inoculation did not affect fungal growth (i.e. mycelial dry weight) in submerged cultures, but essentially blocked aflatoxin biosynthesis in both A. flavus and A. parasiticus. The inhibitory effect was somewhat diminished in heated leaf extracts (Bhatnagar and McCormick, 1988). The inhibitory effect of these extracts was also investigated under in vivo conditions i.e. in developing cotton bolls. Fungal growth in cottonseed was unaffected by the treatments, but the seeds from locules receiving both neem leaf extracts and A. flavus spores simultaneously exhibited one-sixth the inhibition of aflatoxin production as compared to the treatment where the locules received the A. flavus spores 48 hours after the neem extract was injected into the locules. It appears, therefore, that neem leaf extracts contain an aflatoxin inhibiting factor. However, this factor may need to translocate from the fibrous locule surface of the cotton boll to the seed, prior to fungal mycelia, for maximal effect (Zeringue and Bhatnagar, 1989; 1990).

It was earlier observed (Bhatnagar et al, 1988) that the inhibitory activity of the neem leaf extracts on aflatoxin production by the fungi was considerably reduced after heating the leaf extracts by either boiling the leaves in the buffer or autoclaving the leaf extracts (blended and heat-extracted neem leaf preparations). The differences in the volatile components of the blended and the autoclaved, heated neem leaf extracts was examined. The major volatile component present, 3-methyl-2-buten-1-ol, was nearly 400-fold greater in the blended extract than in the heated extract. It has been demonstrated (Zeringue and McCormick, 1988) that a similar compound, 3-methyl-1-butanol, one of the main characteristic odors of living colonies of A. flavus and also a volatile component of cotton leaves, inhibited A. flavus growth by 20% but increased production of aflatoxin B<sub>1</sub> by two fold. Volatiles from blended neem leaf extracts, however, did not inhibit or stimulate either fungal growth or aflatoxin production in A. flavus (this study) or A. parasiticus (Bhatnagar and McCormick, 1988), even though the amount of inoculum and neem extract added in the bioassay was in the same ratio as that added in submerged cultures.

To elucidate the factor(s) responsible for the inhibition of aflatoxin synthesis by neem leaf extracts, the effect of the aqueous leaf extracts on the regulation of secondary metabolism was investigated. The ability of resting mycelia to carry out secondary biosynthesis was used as a test system to monitor the effects of leaf extracts on the process in A. flavus (this study) and A. parasiticus (Bhatnagar and McCormick, 1988; Cleveland et al., 1990). The results demonstrated that aflatoxin biosynthesis was irreversibly inhibited in the mycelia of both fungi by neem leaf constituents; removal of mycelia from exposure to leaf extracts did not restore aflatoxin synthesis. Enzymes required for aflatoxin biosynthesis were, however, apparently intact in the treated mycelia because all the aflatoxin precursors [norsolorinic acid, averantin, versicolorin A, sterigmatocystin and O-methysterigmatocystin (Bhatnagar et al., 1987)] when fed to the treated mycelia were converted to aflatoxin B<sub>1</sub> with nearly the same efficiency as control mycelia not exposed to leaf extracts. Metabolite precursors of aflatoxin biosynthesis were also not detected in mycelial extracts of treated mycelia, but these compounds were present in extracts of the control mycelia. Therefore, neem leaf extracts inhibit aflatoxin biosynthesis by preventing the production of secondary metabolite rather than the regulation of the synthesis or inactivation of enzymes specific to secondary metabolism. This inhibition of aflatoxin biosynthesis in fungal cells apparently occurs at a very early stage of the biosynthetic pathway because (1) norsolorinic acid, a very early precursor (Bhatnagar et al, 1987), was not detected in the treated mycelia; and (2) once the secondary metabolism is initiated, the inhibitory effect of the neem leaf constituents was lost (Bhatnagar and McCormick, 1988).

In conclusion, non-volatile, somewhat heat labile neem leaf constituents irreversibly and almost totally inhibit aflatoxin biosynthesis in A. flavus and A. parasiticus, without affecting fungal growth, in both in vitro and in vivo conditions. Inhibition of aflatoxin biosynthesis appears to occur in the early stages of the biosynthetic pathway. Experiments are underway using individual, separated components from neem leaf extract in order to determine the component(s) responsible for the bioactivity described. Recently, we received a small sample of Margosan-O<sup>TM</sup> from Mr. R. O. Larson, Vikwood Botanicals Ltd., Wisconsin and have tested the efficacy of this product in aflatoxin inhibition in Aspergillus flavus-infected cottonseeds (Zeringue and Bhatnagar, 1990). Margosan-O<sup>TM</sup> is an ethanol extract concentrate obtained from the neem seeds. The commercial extract contained 3000 pm (+ 10%) azadirachtin, a tetranortriterpenoid pesticide which occurs naturally in seed kernal. Margosan-O significantly inhibited aflatoxin synthesis in developing cotton bolls, 8581 ppb aflatoxin was detected in uninoculated, control seeds and 351 ppb in inoculated, treated seeds. These results correlate well with our results with the neem leaf extracts suggesting that azadirachtin or a similar compound in neem leaf extracts may be inhibiting aflatoxin biosynthesis in both A. flavus and A. parasiticus. If the inhibitory factor in neem leaf extracts or Margosan-O could be effective in field studies, these natural products could be used in controlling the preharvest aflatoxin contamination of food and feed commodities.

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#### REFERENCES

- Adye, J. and R. I. Mateles. 1964. Incorporation of labelled compounds into aflatoxins. *Biochim. Biophys. Acta* 86:418-420.
- ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. 1984. In: Official Methods of Analysis, Arlington, VA. 14th ed. 26.052-26.060.
- Bhatnagar, D., T. E. Cleveland and E. B. Lillehoj. 1989. Enzymes in late stages of aflatoxin B<sub>1</sub> biosynthesis: strategies for identifying pertinent genes. *Mycopathologia*. 107:75-84.
- Bhatnagar, D. and S. P. McCormick. 1988. The inhibitory effect of neem (Azadirachta indica) leaf extracts on aflatoxin synthesis in Aspergillus parasiticus. *J. Am. Oil Chem. Soc.* 65:1166-1168.
- Bhatnagar, D., S. P. McCormick, L. S. Lee and R. A. Hill. 1987. Identification of O-methylsterigmatocystin as an aflatoxin B<sub>1</sub> and G<sub>1</sub> precursor in Aspergillus parasiticus. *Appl. Environ. Microbiol.* 53:1028-1033.
- Cleveland, T. E., D. Bhatnagar, P. J. Cotty and H. J. Zeringue. 1990. Aflatoxin reduction - a molecular strategy. *Proc. of The International Biotechnology and Food Safety Symposium*, College Park, Maryland (Oct 10-12, 1989) (in press).
- Detroy, R. W., E. B. Lillehoj and A. Ciegler. 1971. Aflatoxin and related compounds. In: A. Ciegler, S. Kadis, S. J. Ajl, eds. *Microbial toxins* Vol. 6, New York: Academic Press. pp. 3-178.
- Groopman, J. D., R. G. Croy and G. N. Wogan. 1981. In vitro reactions of aflatoxin B<sub>1</sub>-adducted DNA. *Proc. Natl. Acad. Sci. USA.* 78:5445-5449.
- Jacobson, M. (ed) 1989. *Phytochemical Pesticides. Vol. I. The Neem Tree*. CRC Press Boca Raton, Florida. pg. 178.

Lee, L. S., P. E. Lacey and W. R. Goynes. 1987. Aflatoxin in Arizona Cottonseed: A model study of insect vectored entry of cotton bolls by Aspergillus flavus. Plant Disease 71:997-1000.

McCormick, S. P., E. Bowers and D. Bhatnagar. 1988. An improved HPLC procedure for determining the profiles of aflatoxin precursors in wildtype and mutant strains of Aspergillus parasiticus. J. Chromatography. 441:400-405.

Singh, U. P., H. B. Singh and R. B. Singh. 1980. The fungicidal effect of neem (Azadirachta indica) extracts on some soil-borne pathogens of gram (Cicer arietinum). Mycologia 72:1077-1093.

Widstrom, N. W. 1987. Breeding strategies to control aflatoxin contamination of maize through host plant resistance. In M.S. Zuber, E. B. Lillehoj, B. L. Renfro, eds. Aflatoxin in Maize: A proceedings of the Workshop, CIMMYT, Mexico. p. 212-220.

Zaika, L. L. and R. L. Buchanan. 1987. Review of compounds affecting the biosynthesis or bioregulation of aflatoxins. J. Food Protec. 50:691-708.

Zeringue Jr., H. J. and D. Bhatnagar. 1989. Inhibition of aflatoxin production in Aspergillus flavus infected cotton bolls after treatment with neem (Azadirachta indica) leaf extracts. Plant Physiol. (Supp.) 89:200.

Zeringue Jr., H. J. and D. Bhatnagar. 1990. Inhibition of aflatoxin production in Aspergillus flavus infected cotton bolls after treatment with neem (Azadirachta indica) leaf extracts. J. Am. Oil Chem. Soc. (in press).

Zeringue Jr., H. J. and D. Bhatnagar. 1990. Efficacy of neem extract (Margosan-0™) on aflatoxin inhibition in Aspergillus flavus-infected cottonseed. Plant Physiol. (Supp) (in press).

Zummo, N. and G. E. Scott. 1989. Evaluation of field inoculation techniques for screening maize genotypes against kernel infected by Aspergillus flavus in Mississippi. Plant Disease 73:313-316.



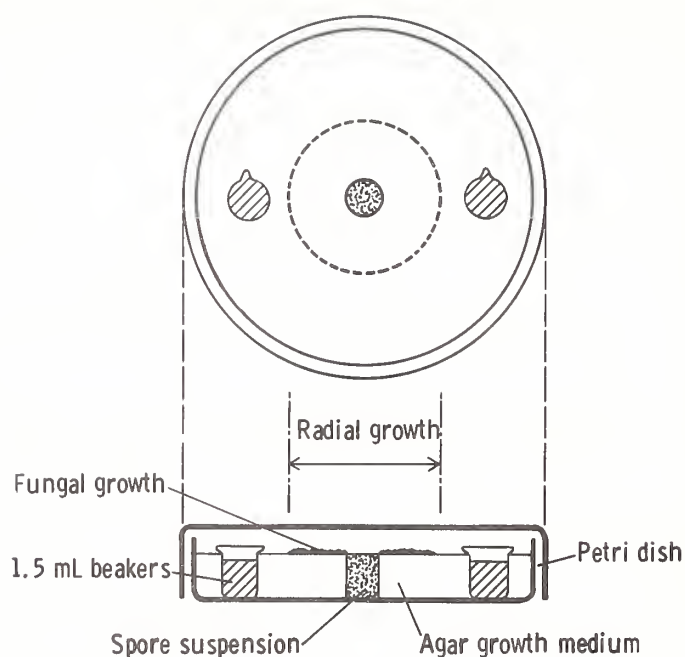


Figure 1. A diagrammatic representation of the experimental set-up for the determination of the effect of volatile component of neem leaves on fungal growth and aflatoxin production on a PDA plate.

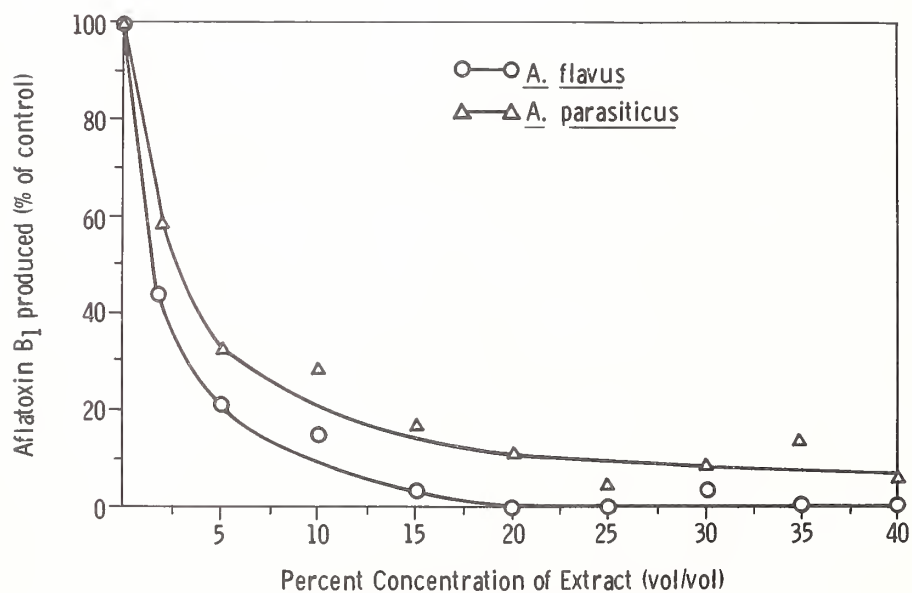


Figure 2. Effect of concentration of neem leaf extracts on aflatoxin synthesis in submerged cultures of *A. flavus* and *A. parasiticus*. 100% refers to 38.4  $\mu$ g aflatoxin B<sub>1</sub> per g mycelial dry weight in *A. flavus* and 28.6  $\mu$ g aflatoxin B<sub>1</sub> in *A. parasiticus*.



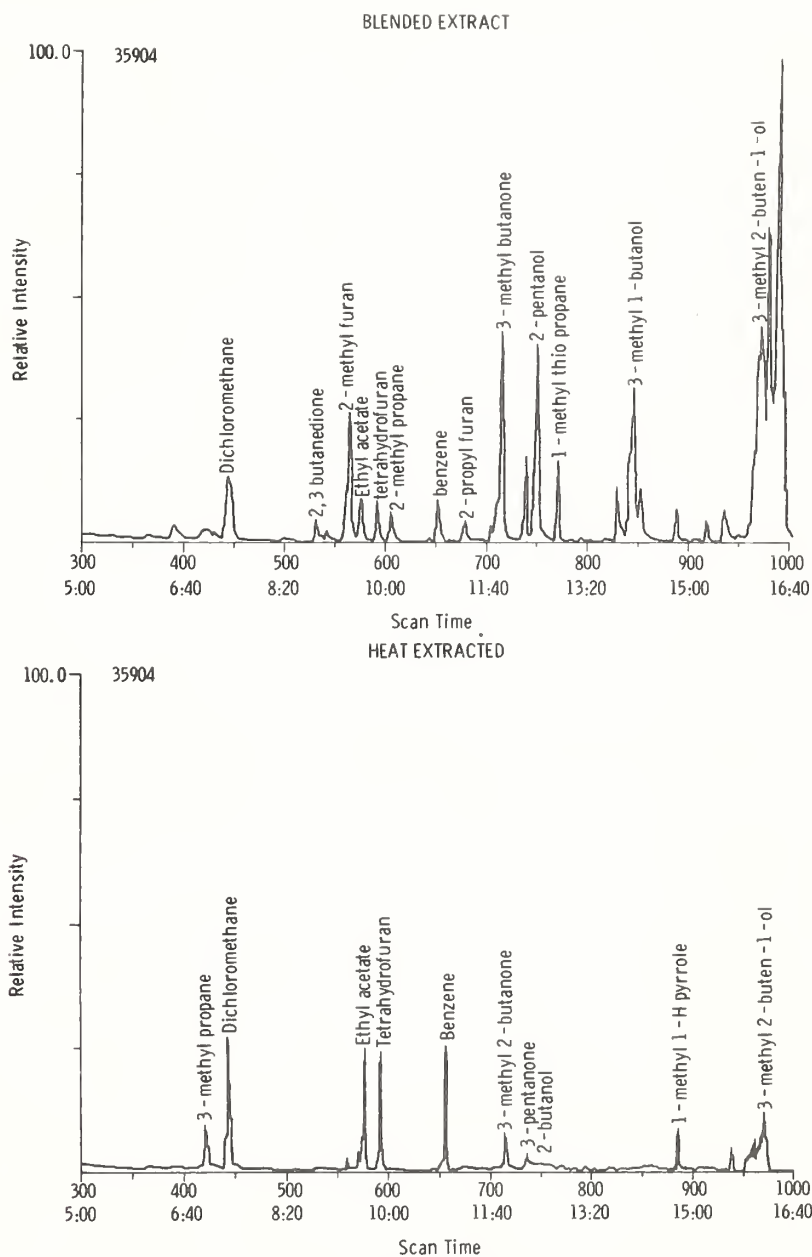


Figure 3. Profile of the major volatile components in blended neem leaf extract (top panel) and the autoclaved, heated extract (bottom panel). The extracts were prepared as described earlier (Bhatnagar and McCormick, 1988). The volatiles were determined as described in Material and Methods.

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ACTIVITY OF NEEM SEED OIL AGAINST GREENHOUSE PESTS,

Hiram G. Larew

ABSTRACT

Several entomologists now agree that aqueous- and alcohol-based extracts of neem seed kernels are useful insecticides; the race is on to develop marketable formulations. Less widely appreciated are the exploitable features of oils extracted from neem seed. This report presents evidence that neem kernel oil is both toxic and repellent to some common greenhouse pests such as the greenhouse whitefly, Trialeurodes vaporariorum (Westwood) and the sweet potato whitefly, Bemisia tabaci (Gennadius). The ovicidal activity of neem kernel oil is also reported.

INTRODUCTION

The insecticidal activity of aqueous and ethanolic neem seed extracts is now firmly established and, as indicated during this workshop, there is currently a great deal of interest in formulating, registering and marketing neem insecticides in the United States. As neem insecticides become mainstream, interest in by-products and wastes of the production process has also increased. The purpose of this paper is to describe attributes of neem seed oil (NSO) (a by-product of the organic solvent extraction process used to produce Margosan-O<sup>TM</sup>) which may be useful in pest management. NSO was kindly provided by Dr. James Walter of W. R. Grace & Co., Columbia, Maryland, U.S.A. Dr. Walter has indicated that NSO is being chemically characterized in his laboratory.

Results are reported in this paper from trials conducted to assess the insecticidal (nymphicidal), repellent and ovicidal activity of NSO. Although trials designed to assess phytotoxicity are not reported herein, observations made recently (Locke and Larew, unpublished data) indicate that at efficacious concentrations NSO is not phytotoxic on a variety of greenhouse plant materials. All trials were conducted in the greenhouse (22-28°C) with chrysanthemums cv. Iceberg as the test plants.

MATERIALS AND METHODS

Nymphicidal Activity. Young plants were exposed to greenhouse whitefly (Trialeurodes vaporariorum (Westwood)) adults for 24 hours so that eggs were laid on the plants. Then, either immediately after exposure (0 days after exposure; 0 DAE) or 4 days after exposure (4 DAE; about one day before egg hatch), the plants were sprayed with either water, 1% or 3% NSO (w/w; no surfactant; 5 plants per treatment). One week after the 4 DAE treatment, when all but a few eggs had hatched on water-sprayed plants, the total number of eggs (hatched or unhatched) and the number of dead, shrivelled eggs and nymphs were counted on 3 middle leaves from each plant. The percent mortality (number of dead eggs and nymphs divided by the total number of eggs) was calculated.

Larew is Biotechnology Specialist, Office of the Science Advisor, Room 320, SA-18, U.S. Agency for International Development, Washington, D.C. 20523. This work was conducted while he was a Research Entomologist, Florist and Nursery Crops Laboratory, Beltsville Agricultural Research Center, U.S. Department of Agriculture, Beltsville, MD 20705.

Repellency. Choice and no-choice repellency trials were conducted. For choice trials, young plants (3 per treatment) were sprayed with either water, 1% or 3% NSO, were allowed to dry, and then were exposed as a group to either greenhouse whiteflies or to sweet potato whiteflies (*Bemisia tabaci* (Gennadius)). Egg counts from 3 leaves per plant were used as an indication of repellency, i.e., greater repellency results in fewer eggs laid.

For no-choice experiments plants were separated by treatment (water or 2% NSO), and then were placed in plexiglass cages (5 plants per treatment) with approximately 100 adult greenhouse whiteflies for 24 hours, and the number of eggs on each plant was counted the following day.

Residual repellency was also tested. Several plants were sprayed (water, 1% or 3% NSO), and were then exposed to greenhouse whiteflies 1, 3 and 7 days after spraying (choice conditions, 4 plants per treatment per exposure date). After exposure eggs were counted on two middle leaves from each plant.

Ovicidal Trials. Ovicidal trials were conducted using NSO on a variety of insect eggs. Young eggs (less than 24 hours old) were sprayed with water, 0.3%, 0.7% or 2% (wt/wt) aqueous mixtures of NSO. Ivory Liquid was added to all oil solutions at a rate of three drops per 100 g of mixture to enhance suspension of the oil. Eggs were incubated on moist filter paper in a petri dish at  $23^{\circ}\text{C} \pm 2^{\circ}$  under a 14L:10D photoperiod. Two days after the last hatching in the controls, all eggs were scored as hatched or unhatched. Egg mortality was corrected with Abbott's formula. Most trials were conducted twice with 100-200 eggs per trial.

## RESULTS

Nymphicidal Activity. (Table 1) In addition to causing some egg death (see ovicidal trials), egg-applied NSO also caused nymphal death soon after emergence from the egg case. Mortality was greatest (99%) when the higher concentration was sprayed on older eggs.

Table 1

Effect of Neem Seed Oil When Sprayed on New and 4-Day-Old Greenhouse Whitefly Eggs Laid on Chrysanthemums			
Treatment	Total Eggs*	Dead Eggs and Nymphs*	% Mortality**
Water	317ab	2c	<1
1%, 0 DAE	185b	100bc	54
3%, 0 DAE	153b	143b	93
1%, 4 DAE	198ab	180b	90
3%, 4 DAE	360a	358a	99

\* Values are means per 100 cm<sup>2</sup> leaf area.  
Means within trial followed by the same  
letter are not significantly different;  
DMRT, P = 0.05, N = 15 leaves.

\*\* Number of dead eggs and nymphs divided by  
the number of eggs.

Repellency. Under choice conditions the number of greenhouse whitefly eggs was reduced by 90% (1% NSO) to 97% (3% NSO). Results from choice experiments using B. tabaci (Table 2) indicate similar levels of repellency. Under no-choice conditions, egg laying by greenhouse whiteflies was reduced by 92% (2% NSO). Repellency was effective 7 days after spraying (Table 3).

Table 2

Repellency of Neem Seed Oil Against <u>Bemisia tabaci</u> on Chrysanthemums	
<u>Treatment</u>	<u>Eggs*</u>
Water	110.0a
1%	18.0b
3%	0.0b

\* Values are means calculated per 100 cm<sup>2</sup> leaf area. Means followed by the same letter are not significantly different; P = 0.05, N = 9 leaves.

Table 3

Neem Seed Oil -- Residual Effects			
<u>Treatment</u>	<u>Eggs*</u>		
	<u>Day 0</u>	<u>Day 3</u>	<u>Day 7</u>
Water	506a	844a	405a
1%	69b	107b	14b
3%	18c	17b	1b

\* Mean number of eggs per 100 cm<sup>2</sup> leaf area. Means in same column followed by the same letter are not significantly different; DMRT, P = 0.05, N = 8 leaves.

Ovicidal Activity. NSO was toxic to two-spotted spider mites (38%, 73% and 87% mean mortality with respective increasing oil concentrations) (Tetranychus urticae (Koch)), and the tobacco hornworm (3%, 28% and 86%, respectively) (Manduca sexta (L.)). In a single trial, mortalities of lacebug (Corythuca cidoniae (Fitch)) eggs were 5%, 8% and 66%, respectively. Neem oil was less toxic to eggs of the other species. For example, at 2%, it caused 41%, 36% and 37% mean mortality in eggs of the Colorado potato beetle (Leptinotarsa decemlineata (Say)), housefly (Musca domestica (L.)) and greenhouse whitefly, respectively. Thus at 2%, neem oil may be a useful ovicidal product against certain insects and mites, but is less useful against others.

In summary, as with other types of oils (Baxendale and Johnson, 1988; Butler et al, 1989; Larew and Locke, in press) neem seed oil holds promise as an insecticide, ovicide and repellent. The fact that NSO demonstrates fungicidal activity (Locke, in these proceedings) suggests that an effective and presumably safe by-product of Margosan-0<sup>TM</sup> production may itself hold considerable promise as a general plant protectant.

#### ACKNOWLEDGEMENTS

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#### REFERENCES

- Baxendale, R. W. and W. T. Johnson. 1988. Evaluation of summer oil spray on amenity plants. *J. Arboriculture* 14:220-225.
- Butler, G. D., Jr., D. L. Coudriet and T. J. Henneberry. 1989. Sweet potato whitefly: Host plant preference and repellent effect of plant-derived oils on cotton squash, lettuce, and cantaloupe. *Southwest Entomol.* 14:9-16.
- Larew, H. G. and J. C. Locke. Repellency and toxicity of sunspray 6E Plus horticultural oil against greenhouse whiteflies. *HortSci.* In press.



## ACTIVITY OF EXTRACTED NEEM SEED OIL AGAINST FUNGAL PLANT PATHOGENS

James C. Locke

## ABSTRACT

Extracts containing triterpenoid compounds from seeds and leaves of the tropical neem tree, *Azadirachta indica*, are known to have potent pesticidal activities. Similarly, an undefined "neem oil" preparation has been reported to inhibit growth of several genera of plant pathogenic fungi. This presentation will describe the activity of a hydrophobic-solvent extracted neem oil which is substantially free of the triterpenoids azadirachtin and salannin against several plant pathogenic fungi. Foliar applications of neem oil formulated with a suitable emulsifying agent and applied as an aqueous spray at 0.25 to 2.0% (w/w) provided excellent control without plant damage. Efficacy was demonstrated against fungi causing powdery mildew and rust in both greenhouse and field trials.

## INTRODUCTION

Although the literature is burgeoning with documentation on the usefulness of neem tree derivatives for insect pest control and it has been reviewed recently (Schmutterer, 1990; Warthen, 1989), there are relatively few references to activity against fungal plant pathogens. The majority report the usefulness of neem (seed) oil cake amendments to natural field soils in reducing damage caused by soilborne fungal pathogens and plant parasitic nematodes (Khan et al., 1973; Khan et al., 1974a). Several authors report that aqueous extracts of various plant parts inhibit soilborne and foliar fungal pathogens *in vitro* (Khan et al., 1974b; Singh et al., 1980). The oil, derived from seed, has been shown to be the most potentially fungitoxic component, possibly due to its sulfur content (Singh et al., 1980).

Neem product development has centered in this country on extraction, concentration, and formulation of azadirachtin, the insecticidal compound found in highest concentration within the seed. As a result of azadirachtin extraction from seed, a large proportion of organic by-products remain for disposal. These oils, waxes, and seed solids still potentially contain many biologically active compounds. Previous work with a crude ethanolic extract of neem seed had demonstrated inhibition of mycelial growth *in vitro* of two fungal pathogens, *Rhizoctonia solani* and *Fusarium oxysporum* (Locke, 1986). It is the purpose of our current research project to evaluate and screen seed extraction by-products for pesticidal activity. Specifically, this report will address the fungitoxic activity of a hydrophobic-solvent extracted oil which is substantially free of the triterpenoids azadirachtin and salannin.

## MATERIALS AND METHODS

All experiments with bean rust, caused by *Uromyces appendiculatus*, were conducted on dry bean, cultivar Pinto 111, planted four seed/10 cm plastic pot and thinned to the three most uniform plants 5-7 days after planting. Primary leaves, expanded to approximately one-third of full size, were treated by spraying test materials onto both upper and lower surfaces to run-off with a low pressure, hand sprayer. A suspension of urediniospores was atomized onto the dried upper and lower leaf surfaces. After the inoculated leaves had dried, the plants were placed in a dew chamber overnight at 19 C to allow germination and penetration by the pathogen (Staveland, 1983).

The neem oil used in all tests was produced by solvent extracting the hydrophobic oils and fatty acids from ground neem seeds, removing the seed solids from the oil-solvent phase by centrifugation, evaporating off the solvent, and clarifying the oil by filtration. The clarified oil was formulated with an appropriate emulsifying agent in a 9:1 ratio (w/w).

Efficacy of neem oil. The concentration of neem oil required in the aqueous emulsion to achieve efficacy was determined using the bean rust assay. The primary leaves of seven-day-old bean plants were sprayed with test emulsions containing 0.0625, 0.125, 0.25, 0.5, or 1.0% (w/w) neem oil about one hour before inoculation with urediniospores. After one week of incubation in the greenhouse, the number of rust pustules was counted on each of the three replicate plants (6 leaves) and the leaf area of each determined to calculate the pustule density (pustules/10 cm<sup>2</sup>). Each of the treatments was compared to the water treated control.

Comparison with a standard fungicide. Three concentrations of neem oil (0.25, 0.5, and 1.0% w/w) in aqueous emulsion were compared to a standard EBDC fungicide using the bean rust assay. The primary leaves of six-day-old bean plants were sprayed with either neem oil emulsion, or the labeled rate of the EBDC fungicide. After one week of incubation in the greenhouse, the number of rust pustules was counted on each of six replicate plants (12 leaves) and pustule densities determined and corrected for leaf area. Efficacy of the neem oil treatments was compared to the EBDC fungicide standard.

Timing of neem oil application. A 1.0% aqueous neem oil emulsion was applied to primary bean leaves just prior to inoculation with bean rust, or at 24 or 48 hr after inoculation. Three plants (6 leaves) received each treatment and pustule density evaluation was made after one week of incubation in the greenhouse. Treatments were compared with each other and against the water treated control to determine protective and/or curative activity of the neem oil.

Protectant activity against powdery mildew. A 1.0% (w/w) aqueous emulsion of unclarified neem oil was applied to the foliage of hydrangea (*Hydrangea macrophylla*) at 14-day intervals. The plants were rooted cuttings in 4-inch pots which were maintained in the greenhouse for approximately four months. Powdery mildew (*Erysiphe polygoni*) occurred naturally and continued to develop on the water treated controls for the duration of the test. After eight applications of neem oil, the plants were destructively sampled, counting the number of leaves mildewed on each plant. No attempt was made to quantify the amount of mildew on each leaf.

Curative activity of neem oil against mildew. A 1.0% aqueous neem oil emulsion was applied to common lilac (*Syringa vulgaris*) leaves in an outdoor test within one week of the natural appearance of powdery mildew (*Microsphaera alni*). The appearance and development of the mildew infections were monitored and compared to the progress on water treated control leaves. Neem oil was reapplied to the test leaves on a 14-day schedule for a period of eight weeks.

## RESULTS AND DISCUSSION

Efficacy of neem oil. At concentrations as low as 0.0625% a statistically significant reduction in the number of bean rust pustules was observed, but only at concentrations of 0.25% or greater was maximum efficacy achieved. The values shown in Table 1 are the average of two trials, and they show that there was no statistical difference in efficacy above the 0.25% level of application. It is possible that, with additional formulation studies and neem oil purification, a higher percent control could be achieved to make it comparable to synthetic fungicides. However, these results give impetus to continue evaluating neem oil as a potential fungicide.

Table 1. Effect of neem oil concentration on efficacy against bean rust.

Treatment	Pustules/10 cm <sup>2</sup>	% Control
water	117.4 a*	0
0.25% oil	22.0 b	81
0.50% oil	11.7 b	90
1.00% oil	11.4 b	90

\* Treatments with same letter are statistically similar; DMRT, P = 0.05, N = 6 leaves.

Comparison with a standard fungicide. Table 2 shows that neem oil, at concentrations as low as 0.5%, can be statistically as efficacious as a standard EBDC fungicide. These results are based on the ideal conditions of a greenhouse/dew chamber trial in which spray coverage of the leaf surface is complete and weathering is not a factor. They clearly show the potential of neem oil as a foliar protectant. Follow up trials need to be designed for the field to determine if under variable environmental conditions and sunlight exposure these results can be duplicated.

Table 2. Comparison of neem oil efficacy with a standard EBDC fungicide against bean rust.

Treatment	Pustules/10 cm <sup>2</sup>	% Control
water	140.6 a*	0
EBDC fungicide	7.0 c	99+
0.25% oil	88.3 b	37
0.50% oil	26.0 c	82
1.00% oil	12.2 c	91

\* Treatments with same letter are statistically similar; DMRT, P = 0.05, N = 12.

Timing of neem oil application. The results of this trial indicate that the effect of neem oil in controlling bean rust is mainly one of protective action. Table 3 shows that the efficacy of neem oil is greatly diminished if applied just 24 hr after inoculation and is completely eliminated if 48 hr elapse before application. Before abandoning the possible use of neem oil in a curative manner, additional formulations, rates, and diseases should be evaluated. There is an indication (see below) that neem oil at a concentration of 1.0% can inhibit other fungal pathogens if applied soon after infection occurs.

Table 3. Effect of timing of neem oil application on efficacy against bean rust.

Treatment	Pustules/10 cm <sup>2</sup>	% Control
water	253.2 a*	0
1.0% oil PI	18.1 c	93
1.0% oil 24 hr	175.4 b	31
1.0% oil 48 hr	251.1 a	1

\* Treatments with same letter are statistically similar; DMRT, P = 0.05, N = 6.

Protectant activity against powdery mildew. Table 4 shows that the repeated application of neem oil, at least under greenhouse conditions, can effectively prevent the development of powdery mildew on hydrangea. Because of the slow growth rate of hydrangea in the greenhouse, the 14-day interval of neem oil application could have probably been increased to at least 21 days with the same resultant protection. In addition to providing a protective coating on the leaf surface, the oil also provided a slight leaf shine. No growth reduction was noted even though the repeated applications did result in visible oil/wax residues on the leaf surface.

Table 4. Effect of protectant neem oil sprays on powdery mildew development on greenhouse hydrangeas.

Treatment	Mildew*		% leaves mildew +
	+	-	
water	48.6	57.0	46.0
1% neem	1.8	101.4	1.7

\* Counts are average number of leaves with mildew (+) (5 plants/treatment).

Curative activity of neem oil against powdery mildew. Weekly observations of this trial indicated that when neem oil was applied to incipient powdery mildew infections on common lilac the disease progress was almost completely arrested. Visible mycelium that was sprayed looked debilitated following treatment and there was little or no spread of the fungus from these sites. The follow-up applications (14-day interval) were effective against any small patches of mildew that appeared subsequent to the previous treatment resulting in foliage that was almost disease-free in appearance compared to the water treated control. Additional field trials need to be done to ascertain the effectiveness of neem oil as a curative treatment for powdery mildew.

## REFERENCES

- Khan, M. W., A. M. Khan, and S. K. Saxena. 1973. Influence of certain oil-cake amendments on nematodes and fungi in tomato field. *Acta Bot. Indica* 1:49-54.
- Khan, M. W., A. M. Khan, and S. K. Saxena. 1974a. Rhizosphere fungi and nematodes of eggplant as influenced by oilcake amendments. *Indian Phytopath.* 27:480-484.
- Khan, M. W., M. M. Alam, A. M. Khan, and S. K. Saxena. 1974b. Effect of water soluble fractions of oil-cakes and bitter principles of neem on some fungi and nematodes. *Acta Bot. Indica* 2:120-128.
- Locke, J. C. 1986. In vitro fungitoxicity of an ethanolic extract of neem seed on Rhizoctonia solani and Fusarium oxysporum. *Phytopathology* 76:1129.
- Schmutterer, H. 1990. Properties and potential of natural pesticides from the neem tree, Azadirachta indica. *Annu. Rev. Entomol.* 35:271-297.
- Singh, U. P., H. B. Singh, and R. B. Singh. 1980. The fungicidal effect of neem (Azadirachta indica) extracts on some soil-borne pathogens of gram (Cicer arietinum). *Mycologia* 72:1077-1093.
- Stavelly, J. R. 1983. A rapid technique for inoculation of Phaseolus vulgaris with multiple pathotypes of Uromyces phaseoli. *Phytopathology* 73:676-679.
- Warthen, J. D., Jr. 1989. Neem (Azadirachta indica A. Juss): Organisms affected and reference list update. *Proc. Entomol. Soc. Wash.* 91:367-388.







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